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THE INFLUENCE OF PROLONGED HYPER AND HYPOTHYROID STATES ON ADRENERGIC ACTIVITY IN RATS

BY

© GRANT T. TU

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ABSTRACT

To investigate the possibility that elevated responses to sympathetic activity in hyperthyroidism may be due, in part at least, to altered activity on the neuronal side of neuro-effector junctions, the influence of chronic hyper and hypothyroidism on the catecholamine content of rat tissues and on the accumulation in, and efflux of tritiated noradrenaline (^{3}H-NA) from the heart, brain, adrenal glands, salivary glands and vasa defferentia were studied. Hypothyroidism was induced in the rats by thyroidectomy and hyperthyroidism was induced in thyroidectomized rats by administration of low doses of thyroxine (25 μg/kg/day) for 8 and 12 week periods. Control groups which were similarly housed and fed were sham-operated. The hyperthyroid rats showed no significant difference from the euthyroid control group with respect to the tissue contents of adrenaline, noradrenaline or dopamine, or the accumulation and rates of efflux of ^{3}H-NA. In contrast most of the tissues of the hypothyroid group showed a significantly higher catecholamine content than the corresponding tissues of the euthyroid controls but a lower accumulation and higher efflux rate of ^{3}H-NA from their hearts and adrenal glands. The tissues of the hypothyroid animals were significantly lighter than those of the corresponding euthyroid and hyperthyroid groups but the body weights were also lower so that this tissue weight difference was not sufficient to account for the increased catecholamine content on a μg/g tissue basis. These results indicate that in chronic hyperthyroidism of rats the rate of NA turnover did not change whereas in chronic hypothyroidism it increased. This increase may be due to an increased tyrosine hydroxylase activity and synthesis of catecholamines

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To investigate the possibility that elevated responses to sympathetic activity in hyperthyroidism may be due, in part at least, to altered activity on the neuronal side of neuro-effector junctions, the influence of chronic hyper and hypothyroidism on the catecholamine content of rat tissues and on the accumulation in, and efflux of tritiated noradrenaline (^{3}H-NA) from the heart, brain, adrenal glands, salivary glands and vasa defferentia were studied. Hypothyroidism was induced in the rats by thyroidectomy and hyperthyroidism was induced in thyroidectomized rats by administration of low doses of thyroxine (25 μg/kg/day) for 8 and 12 week periods. Control groups which were similarly housed and fed were sham-operated. The hyperthyroid rats showed no significant difference from the euthyroid control group with respect to the tissue contents of adrenaline, noradrenaline or dopamine, or the accumulation and rates of efflux of ^{3}H-NA. In contrast most of the tissues of the hypothyroid group showed a significantly higher catecholamine content than the corresponding tissues of the euthyroid controls but a lower accumulation and higher efflux rate of ^{3}H-NA from their hearts and adrenal glands. The tissues of the hypothyroid animals were significantly lighter than those of the corresponding euthyroid and hyperthyroid groups but the body weights were also lower so that this tissue weight difference was not sufficient to account for the increased catecholamine content on a μg/g tissue basis. These results indicate that in chronic hyperthyroidism of rats the rate of NA turnover did not change whereas in chronic hypothyroidism it increased. This increase may be due to an increased tyrosine hydroxylase activity and synthesis of catecholamines

due to a reduction in circulating iodotyrosine derivatives, or possibly to stress. This work indicated that the elevated response observed in hyperthyroidism which resembles elevated activity of the sympathetic nervous system, is not due to altered uptake, synthesis or turnover of NA at the neuronal side of the neuro-effector junction.

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To My Parents

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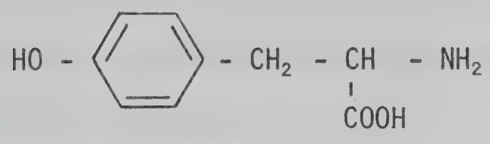
INTRODUCTION

The relationship between thyroid hormone and the catecholamines has been an important subject for investigation since Goetsch (1918) showed that increased sensitivity to adrenaline (A) could be used as a diagnostic test for hyperthyroidism. The resemblance of excessive sympathetic nervous activity to the symptoms of hyperthyroidism, and similarities between the physiological effects of the hormones from the thyroid gland and the adrenal medulla showed a possible relation between thyroid hormone and the catecholamines. Considerable evidence of a relationship between levels of thyroid activity and the sympathetic nervous activity has been accumulated, but the mechanism responsible for the elevated responses to adrenergic stimulation in hyperthyroidism and for the reduced responses in hypothyroidism has not been clarified.

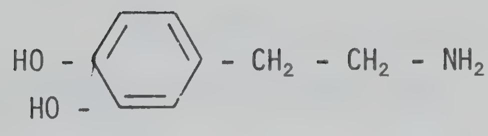
A. Chemical Structures of Thyroid Hormones and Catecholamines

The thyroid hormone, thyroxine, is a derivative of tyrosine, which is also a precursor of the catecholamines and there are therefore some similarities in the chemical structures of thyroid hormones and the catecholamines as illustrated below. There is a possibility that these chemical relationships are responsible for some of the similarities in responses, but we must note that, characteristic of the differences between hormonal and nervous control, A and noradrenaline (NA) are characterized by rapid responses whereas thyroxine induces slower and longer lasting actions. The mechanism of these similarities is not known but is essential for the response to A and NA, and Zaimis et al. (1969) have recently shown

Structural Relationship of the Catecholamines to the Thyroid Hormones



TYROSINE



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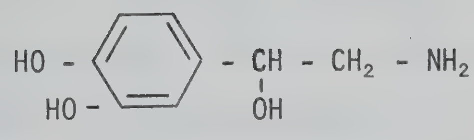

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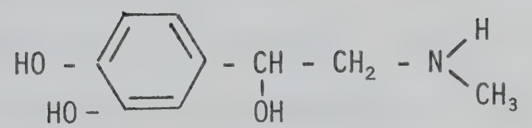
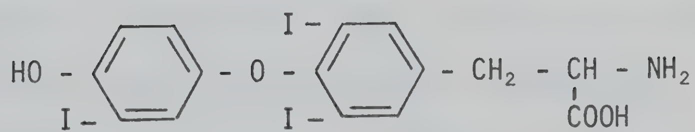
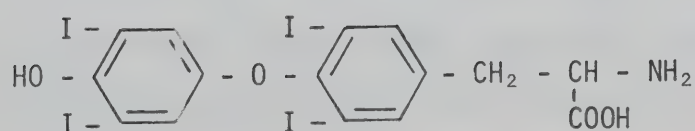


ADRENALINE



TRIIODOTHYRONINE



THYROXINE

that chronic alterations in muscle excitability accompany chronic hyperthyroidism and that these changes probably influence the storage and utilization of calcium.

B. Mechanism of Action of Thyroid Hormone

Hoch (1968) has shown that the thyroid hormones act directly on mitochondria, and thereby control the transformation of energy derived from oxidations into a form utilizable by the cell. Through their direct actions on mitochondria, the hormones also control, indirectly, the rate of protein synthesis and thereby the amount of oxidative apparatus in the cell. In hypothyroidism, slow fuel consumption leads to a low output of utilizable energy. In hyperthyroidism, rapid fuel consumption leads to a high energy output but, in hypothyroidism, as efficiency decreases, the utilizable energy produced also decreases. Tapley (1964) suggested that the mitochondrial membrane may be the primary locus of action of the thyroid hormone. Michels et al. (1963) reported that the treatment of rats with l-thyroxine increased the incorporation in vivo of radioactive amino acids into protein of liver, kidney and heart, but not of spleen, testis or brain. The distribution of the effect among the organs was the same as that observed in thyroxine stimulation of oxidative metabolism. Thus, stimulation of protein synthesis seemed to be a physiological action of the thyroid hormone. Roodyn et al. (1965) demonstrated that triiodothyronine stimulated amino acid incorporation into mitochondrial protein in isolated rat liver. Moreover, Buchanan and Tapley (1966) showed that rat liver mitochondria incorporated amino acids into acid-precipitable material when incubated with

potassium, magnesium, inorganic phosphate and an oxidizable substrate. l-Thyroxine and a number of its analogues, added _in vitro_, markedly stimulated this incorporation. It was suggested that the stimulation of protein synthesis by thyroid hormones _in vivo_ consists of two components: (1) an initial, cytoplasmic, mitochondria-dependent stimulation of the existing protein synthesizing apparatus, followed by (2) a secondary, nuclear-mediated, cellular response or adaptation which leads to an increase in the amount of protein-synthesizing machinery (Sokoloff _et al_., 1968).

Astwood (1970) said that the effect of thyroid hormone in restoring growth in the thyroidectomized animal is one of the most sensitive responses to the thyroid hormone. In thyroidectomized rats, a dose of 0.5 μg of l-thyroxine was required to maintain metabolic rates at levels normal for the age. Red cell volume was normal at 1 and 5 μg, but at the lower doses there was no prevention of the anemia which follows thyroidectomy. Pituitary acidophils were normal at 1 μg whereas the basophils required between 1 and 5 μg for prevention of hyperplasia and hypertrophy (Evans _et al_., 1960a). The latter group (Evans _et al_., 1960b) also studied the growth response of thyroidectomized rats to high levels of iodide. The suggestion was made that iodide itself in high concentrations may exert a thyroxine-like effect on tissues. Moreover, Taurog and Evans (1967) reported that thyroxine was formed at some extrathyroidal site(s) in completely thyroidectomized rats injected daily with 5 mg of iodide. Evans _et al_. (1966) showed that effective doses of iodide presumably resulted in the formation of thyroxine in quantities equivalent to the daily injection of 0.25-0.5 μg. They also showed that propylthiouracil abolished most of the growth response to iodide.

According to a study of Hoch (1965), the efficacy and rapidity of the potentiation of DNP-induced calorigenesis by subcalorigenic doses of l-thyroxine in hypothyroid rats suggested a primary action of l-thyroxine.

C. <u>The Effects of Catecholamines on Uptake, Release and Turnover of Iodine and Thyroid Hormone</u>

According to Hays' study (1965), when A was given 30-60 minutes before radioiodide, uptake was stimulated in normal human thyroid, but at 24 hours uptake had returned to control values. An intravenous infusion of A from 30 to 90 minutes after radioiodide injection caused a significant depression in the rate of thyroidal accumulation of radioactivity during the infusion. Maayan and Ingbar (1968) demonstrated that in isolated calf thyroid cells l-A and NA consistently stimulated the accumulation and organic binding of iodine. The effect was partially inhibited by phentolamine, but not propranolol, and hence may be mediated by alpha receptors. Theophylline did not mimic or enhance the A effect, suggesting that the latter probably did not result from activation of adenyl cyclase.

Ackerman and Arons (1962) reported that inorganic plasma ^{131}I levels increased 60% or more in dogs following A or NA administration. Dogs receiving intravenous injections of thyroid stimulating hormone (TSH), had significant increases in thyroid vein inorganic ^{131}I. The inorganic ^{131}I rise occurred simultaneously with increases in PBI^{131}. Galton (1965) showed that in thyroidectomized rats, the urinary excretion of ^{131}I in the 24 hour period following a single injection of ^{131}I-labelled thyroxine

was increased by adrenaline. Adrenaline and sometimes NA, when administered to mice for 4 days, induced a significant increase in the deiodinating activity of homogenates of mouse liver. They also showed that the deiodinating activity was greatly decreased in tissues obtained from mice pretreated with reserpine.

The experiments of Hays and Solomon (1964) indicated that the rate of disappearance of ^{131}I-labelled thyroxine (T_4-^{131}I) from the serum in euthyroid men decreased during A treatment. Urinary excretion of ^{131}I paralleled plasma T_4-^{131}I level.

D. The Influence of Decreased Amount of Catecholamines on the Effects of Thyroid Hormone

Lövei and Bona (1956) reported that rauwolfia preparations may well be good in hyperthyroidism. In addition, Canary *et al.* (1957) showed that severe hyperthyroidism in man was converted to a mild disease after two to four weeks of treatment with reserpine. However, they concluded that hyperthyroid patients receiving reserpine may not demonstrate the usual clinical manifestations of their disease except for an enlarged thyroid gland.

Ramey *et al.* (1955) proposed that in rats, the sympathetic neurohumors were involved in the expression of at least certain of the actions usually attributed solely to thyroxine. Gaffney *et al.* (1961) studied the effects of guanethidine, a drug that selectively interrupts adrenergic reflexes and produces tissue catecholamine depletion, on triiodothyronine-induced hyperthyroidism in man. After guanethidine was

administered to hyperthyroid patients, the heart rate returned to control levels and palpitation and tremor disappeared, but when guanethidine was withdrawn the tachycardia, palpitation and tremor returned. They suggested that guanethidine may be of clinical value in rapidly counteracting many of the manifestations of the hyperthyroid state. Lee et al. (1962) evaluated the effect of guanethidine on the manifestations of hyperthyroidism in 27 patients. Significant improvements in the cardiovascular, neuromuscular and metabolic manifestations were noted within the first week of treatment, and their results provide evidence of the physiological interactions between the sympathetic catecholamines and the thyroid hormones. Barker and Makiuchi (1965) showed that guanethidine and bretylium did not eliminate the peripheral effects of thyroxine in rats, although they may diminish responses. Goldstein and Killip (1965) suggested that guanethidine may be useful as a short-term aid in the occasional patient with severe heart failure and thyrotoxicosis. A note of caution was sounded, however, since it was possible that guanethidine may reduce blood flow to some vascular beds out of proportion to change in oxygen demand.

Wilson et al. (1964) studied the pharmacodynamic effects of beta adrenergic receptor blockade in patients with hyperthyroidism. Despite inhibition of the chronotropic and inotropic effects of isoproterenol, nethalide did not significantly change oxygen consumption, heart rate, cardiac output, and systemic mean arterial or mean right atrial pressures in these patients. Their findings did not support the hypothesis that the hemodynamic changes in hyperthyroidism were mediated through adrenergic stimulation of beta adrenergic receptors. Theilen et al. (1964) reported that triiodothyronine-induced hypermetabolic subjects and normal subjects did

not differ in their circulatory responses to mild exercise and that beta receptors were not essential for the hemodynamic changes.

E. The Influence of Stress on Thyroid Function

1. Emotional changes

Brown-Grant *et al*. (1954) reported that emotional stress produced in rabbits by subcutaneous faradism, restraint, or abrupt changes in environmental lighting induced a prompt inhibition, of 1-2 days' duration, of the release of ^{131}I-labelled hormone from the thyroid gland. Similar effects on thyroid activity were also produced by physical traumata (haemorrhage, surgical operations, injection of turpentine) but neither denervation of the thyroid gland (stellate ganglionectomy) nor adrenalectomy prevented the inhibition of release of thyroid hormone that follows emotional stress. Thus, they suggested that the central nervous system could influence thyroid activity, and that this influence was mediated through alterations in the secretion of TSH from the anterior pituitary gland. The possibility that emotional disturbances, acute or chronic, acting through the hypothalamus to produce a hypersecretion of TSH, may play a part in the etiology of Graves' disease was suggested by Brown-Grant (1960). Volpé *et al*. (1960) demonstrated the effect of physical and emotional tensions and strains on the fluctuation of the serum protein bound iodine level in groups of healthy people. The effect of the tension and strain of examination was observed in 11 medical students preparing for annual examinations and in 11 Royal College of Physicians candidates. Also, the stress of athletic contest on the protein bound iodine level was

observed in 7 professional football players in training and during their scheduled games. Falconer and Hetzel (1964) studied the effect of emotional stress and TSH on thyroid vein hormone level in sheep with exteriorized thyroid. The results showed rises in PBI^{131} and PBI 15-30 minutes after insertion of the cannula into the jugular vein. Subsequently, after these changes had subsided, similar rises were demonstrable after a series of firework explosions, and most consistently, after exposure to a barking dog. These rises lasted up to 2 hours. Restraint was followed by an increase in PBI^{131} and similar but larger rises in PBI^{131}, PBI and also free ^{131}I followed both intramuscular and intravenous administration of TSH.

2. Muscular exercise

Bondy and Hagewood (1952) showed that plasma protein bound iodine concentration of the intact rat was decreased after prolonged fasting, exhausting exercise and exposure to cold. This effect was not merely a result of suppressed thyroid function, since exercise and cold stress increased the rate of disappearance of injected thyroxine in thyroidectomized rats. Lashof *et al*. (1954) demonstrated that muscular exercise in human subjects produced no alteration in peripheral utilization of thyroid hormone as measured by concentration of circulating hormone and by rate of disappearance of injected radiothyroxine. Furthermore, studies on the rate of thyroxine secretion (T.S.R.) in the horse, as measured from degradation of labelled thyroxine, were reported by Irvine (1967). In the resting horse, the mean T.S.R. was 0.49 mg/453.6 kg (1000 lb), with a mean value of PBI of 1.80 μg/100 ml, volume distribution of 60 liters, and a half-life of 2.31 days. Partly and fully trained horses showed increases

of 38% and 65% in T.S.R. associated with lower values for PBI and greatly decreased half-life. Nayer et al. (1968) demonstrated that in 11 trained male athletes (20-24 years old) muscular exercise was associated with a decrease in the circulating free thyroxine level. And they suggested that an increase in the cellular utilization of thyroxine was a possible explanation of these findings. Similar observations were made by Irvine (1968). When non-athletic young men commenced taking daily muscular exercise (running) peripheral degradation of thyroxine increased after a latent period, reaching 40% above resting level after 6 days. Athletes in moderately severe training had a thyroxine degradation/secretion rate 75% above that of resting, non-athletes. This fell significantly after 3 days' rest. PBI and free thyroxine levels were not significantly changed, the increase being primarily due to increased peripheral deiodination of thyroxine, which is believed to be due to repeated muscular exercise *per se*.

3. Temperature

In experimental animals, a fall in temperature accelerates thyroid function and a rise in temperature depresses it. These changes may be responses to altered caloric requirement for maintenance of a stable body temperature. The response to cold is especially marked; it can be identified by increased size of the thyroid as well as by more rapid turnover of ^{131}I in the gland. Returning the animals from a low to normal temperature promptly restores normal thyroid function. It was suggested that, at least in animals, increased loss of thyroxine in the feces may occur in cold environments and contribute to thyroid activation (Means

et al., 1963).

4. Surgery, disease, toxin and other stressful stimuli

Fore *et al.* (1966) demonstrated that during the investigation of the influence of stressful stimuli on thyroxine metabolism, ethyl ether anesthesia administered during surgery has caused rapid redistribution of peripheral thyroxine. Since ether anesthesia causes a rapid shift of tissue thyroxine into the serum without measurable changes in serum binding, they suggested that tissue binding of thyroxine was the factor which determines the serum protein bound iodine at any given level of serum binding capacity. Ether anesthesia might alter tissue binding acutely, and it was probable that other stressful stimuli might do the same.

Sterling and Chodos (1956) reported that extrathyroidal organic iodine in humans was diminished in myxedema and increased in thyrotoxicosis, as compared with the normals, and that the turnover rates of extrathyroidal hormone were slower than normal in myxedema and accelerated in thyrotoxicosis. They also showed that hypermetabolic subjects without endocrine disease, but with leukemia and fever, had increases in thyroxine pools, turnovers and degradation rates, although these were less pronounced than in thyrotoxicosis.

The effect of bacterial exotoxins on thyroid function of various animal species has been studied by Gerwing *et al.* (1958). It was shown that a single injection of toxin depressed the rate of release of ^{131}I from the thyroid glands of rats, mice and rabbits, but increased the rate of release in guinea pigs and Rhesus monkeys. A single injection

of TSH stimulated thyroid function in rats, mice and rabbits, the activity of whose glands had been depressed by toxin. Moreover, Gerwing (1958) studied the effect of continuous low grade toxic stress on thyroid function of rats and guinea pigs. The results indicated that such stimulus in rats caused primarily a depression in thyroid function followed by a period wherein thyroid function appeared to be normal, and terminating in a phase of thyroid overactivity. Continuous toxic stress in the guinea pig caused increasing thyroid overactivity throughout the experimental period.

The responses to various stress stimuli (noise, cold, tissue damage, adrenaline, pitressin, typhoid vaccine, diphtheria toxin, electroconvulsions) were investigated by Brown-Grant and Pethes (1960). In each case a decrease in the rate of release of ^{131}I from the thyroid was observed. Thus, they concluded that the guinea pig responded to acute stress with a prompt but reversible decrease in thyroid activity, as had been observed in other species.

F. The Effects of Thyroxine on Synthesis, Uptake and Release of Noradrenaline

The enzyme, tyrosine hydroxylase, which catalyzes the initial step in the formation of NA from the dietary precursor tyrosine, is inhibited, *in vitro*, by 3-iodo-l-tyrosine. Goldstein *et al*. (1965) have shown that 3-iodo-l-tyrosine inhibits endogenous catecholamine biosynthesis in rats. As iodotyrosine is found in the peripheral blood of thyrotoxic or normal humans, Goldstein *et al*. suggested that there was the question whether thyroid hormones affect the biosynthesis of catecholamines *in vivo*. Recently, Lipton *et al*. (1968) showed marked accelera-

tion of the synthesis of ^{14}C-NA from ^{14}C-tyrosine in the heart and adrenal of the hypothyroid rats. Landsberg and Axelrod (1968a) demonstrated that thyroid deficiency is associated with an increase in cardiac NA turnover in rats; they (1968b) also showed the reduced accumulation of ^{3}H-NA in the rat heart following hypophysectomy. In the study of turnover of NA in thyrotoxic and nonthyrotoxic mice, Beaven _et al_. (1963) reported that NA pool size and rate of synthesis were normal in thyrotoxicosis. On the other hand, Prange _et al_. (1970), after measuring the synthesis of NA from tyrosine in the heart and brain of hyperthyroid rats, concluded that hyperthyroidism probably decreases the release as well as the synthesis of NA.

Dengler (1961) found that heart slices from hyperthyroid rats contained less ^{3}H-NA after incubation than those from controls. It was concluded from this observation that thyroxine, like cocaine, inhibited the uptake of catecholamines by the heart. Margolius and Gaffney (1964 and 1965) reported that the atrial and aortic NA concentrations were reduced below control levels in both the hypothyroid and hyperthyroid animals. Therefore, they suggested that the size of the sympathetic transmitter store was under the influence of thyroid hormone.

G. The Influence of Different Thyroid States on the Effects of Catecholamines.

1. Changes in hemodynamics

Raab (1944) demonstrated that the tolerance of the heart to A was found to be distinctly lowered by pretreatment with thyroxine and distinctly elevated by pretreatment with thiouracil. Hoffman *et al.*(1947) showed that the hearts of hyperthyroid rats had a high sensitivity to A but those of hypothyroid animals had a much lower sensitivity to A. According to Schneckloth *et al.* (1953), in patients with thyrotoxicosis a striking diminution in pressor responsiveness to NA was observed. On the contrary, in patients with long-standing myxedema, administration of l-thyroxine was associated with a marked potentiation in the systolic pressor responsiveness to NA. Smith (1954) showed that thyroxine immediately and directly increased the sensitivity of the isolated swine carotid arteries to l-adrenaline. Furthermore, thyroxine prolonged the constriction of the vasa vasorum produced by l-A over 10 times, although the hormone alone did not cause constriction of either the arterial wall or the vasa vasorum. After the rate of contraction of isolated rat atria were modified by changes in temperature and by the addition of A, the experiments of Thier *et al.* (1962) indicated that atria from athyroid rats contracted slowest and those from thyroxine treated rats fastest. Atria from hyperthyroid rats accelerated faster than those from athyroid or euthyroid rats in response to warming from 28° to 35°C and to adrenaline. The maximum atrial rates obtained by warming and by adding A varied directly with the level of the thyroid activity. In addition, Leak and Lew (1963) reported that treatment with

thyroxine did not appear to increase the cardiovascular sensitivity to A except in one patient treated to the stage of hyperthyroidism, suggesting that the synergism between thyroid hormone and the catecholamines may exist only at abnormally high levels of thyroid hormone activity. The circulatory response to insulin-induced hypoglycemia was increased by the correction of hypothyroidism, probably as a result of increased A secretion from the adrenal medulla.

2. Changes in metabolism

Horstmann (1954) reported that subcutaneous injection of A in hyperthyroid patients was followed by an increased oxygen intake, which was larger and reached its maximum sooner than in normals. This abnormal reaction disappeared after thyroidectomy. Swanson (1956) showed that thyroidectomy inhibited, and thyroxine potentiated, the calorigenic effect of adrenaline. The oxygen consumption of thyroidectomized rats varied linearly as the log dose of thyroxine. Further studies were done by this author (1957) which showed that, after oxygen consumption at 30°, 18° and 10°C was measured in several groups of thyroidectomized rats, maintained on fixed levels of thyroxine, the metabolic rate varied inversely as the temperature. Thus, it was postulated that the main role of thyroxine in cold acclimatization was the potentiation of the calorigenic action of endogenous adrenaline.

The dependence of the lipolytic action of A upon thyroid hormone, was studied on rat epididymal adipose tissue *in vitro* by Debons and Schwartz (1961). In the case of fat pads removed from hypothyroid animals, no increase in the rate of release of free fatty acids (FFA)

was observed after A; in the case of fat pads removed from hyperthyroid animals, the A-induced release of FFA was markedly exaggerated. This thyroidal enhancement of A action on the fat pad was maximal in tissues removed after 15 hours from animals that had received repeated intraperitoneal injections of triiodothyronine. These studies showed that the thyroid hormone was essential for the A-induced release of FFA from adipose tissue. Harlan et al. (1963) demonstrated that in hyperthyroid patients there was a significant elevation of resting levels of FFA and an enhanced mobilization of FFA after catecholamine stimulation. Hypothyroid subjects presented a wide variation in fasting levels and a broad range of response to catecholamine stimulation. Mobilization of FFA after catecholamine infusion was decreased, and this diminished response appeared to be related to the degree of thyroid deficiency. It was suggested that thyroid hormone has an effect on utilization as well as mobilization of fatty acids. Bray and Goodman (1965) showed that the heart rate and the lipolytic effect of A on adipose tissue were increased 3 hours after giving 45 μg of triiodothyronine intravenously and 6 hours after giving 15 or 45 μg of triiodothyronine subcutaneously.

The excess of thyroid hormone enhanced the hyperglycemic effect of A in rats (Gellhorn and Feldman, 1941) and in rabbits (Trendelenburg, 1953), as the result of potentiation of rat liver (Leonard and Ringler, 1954) and rat muscle glycogenolysis (Hornbrook and Brody, 1963).

H. Relation Between Response to Catecholamines and Thyroid States

Many studies point to a relationship between the level of thyroid activity and the degree of response to exogenous or endogenous catecholamines, a phenomenon which has been studied (Waldstein, 1966) in intact animals and man, and in isolated tissues and organs.

The chronotropic and inotropic cardiac responses to both A and NA are augumented when excess thyroid hormones are present (Brewster et al., 1956) and, in hypothyroidism, reductions of the chronotropic responses have also been reported. For example, Hill and Turner (1968) have reported that the mean heart rate of hyperthyroid patients was significantly higher than that of normal subjects, while that of hypothyroid patients was lower. In addition Harrison (1964) has reported that thyroid hormones sensitize animals to the cardiovascular, glycogenolytic, and lipolytic effects of A and NA. However, Harrison et al. (1967) also reported that uncomplicated primary hyperthyroidism is not accompanied by an obligatory increase in activity of the sympathetic nervous system, and they suggested a sensitization of the cardiovascular and metabolic response mechanisms to normal catecholamine levels occurs in hyperthyroidism. Swanson (1956) reported that hypothyroidism was associated with poor response to catecholamines, but, on the other hand, Margolius and Gaffney (1965) reported that treatment of dogs with thyroid hormone or ^{131}I to render them hyperthyroid or hypothyroid, did not affect their arterial pressor or chronotropic cardiac response to either endogenously released or exogenously administered NA.

I. NA Content in the Tissues in Relation to Thyroid States

No direct correlations between heart muscle catecholamines and thyroid hormone have been established though a number of studies have been made on the role of catecholamines in hyperthyroidism and in hypothyroidism. However, evidence has accumulated that the effects of the thyroid hormone on myocardial function are mediated in part through the action of NA and A (Kurland et al., 1963).

In earlier studies, Leduc et al. (1955) found myocardial NA diminished and A increased following administration of thyroid hormone to rabbits. Conversely, both Goodall (1951) in sheep, and Hökfelt (1951) in rats, observed an increase in myocardial NA during thyroxine treatment, while thyroidectomy was followed by diminution of NA and increase of A, or no significant changes. Raab (1948) stated that myocardial catecholamines were unchanged or slightly increased in thyrotoxicosis.

In recent studies, Kurland et al. (1963) showed that in thyrotoxicosis, atrial A and NA concentrations were decreased, ventricular A was unchanged and ventricular NA was decreased in rabbits. They also found that in hypothyroidism, atrial A and NA were decreased and ventricular A was unchanged, but in the hypothyroid ventricle, NA content was markedly increased. Wurtman et al. (1963) reported that cardiac NA content in rats decreased in hyperthyroid state. Kandror et al. (1967) obtained the result that A and NA content in myocardium of the left ventricle as well as the metabolites of A and NA in 24 hour urines of rabbits given high thyroid hormone decreased, but the dopamine content was elevated. Thyroidectomy resulted in a statistically significant increase in the hypothalamic NA

content; and at the same time, increased levels of NA were noted in the cerebral hemispheres. On the other hand, severe thyrotoxicosis resulted in diminished NA levels in both areas of the brain (Nahnybida, 1967). On the contrary, Goodkind et al. (1961) found a decrease in atrial NA in hypothyroid guinea pigs. They reported an increased NA content in thyrotoxic atria. Lee et al. (1965) showed that the atria isolated from rabbits injected with thyroxine had a faster rate of beating than those from normal rabbits, and the NA content of the heart was increased. Yamagata et al.(1963) however, reported that NA content was not significantly changed in hyperthyroidism though there was a tendency to decrease in hypothyroidism in rats. Varma et al. (1963) found that thyroidectomy greatly reduced the incidence of ventricular fibrillation without significantly reducing cardiac catecholamines.

Kurland et al. (1963) stated that the methodology utilized in some earlier studies was currently acknowledged to be inadequate for accurate measurement of NA and A, and might have been responsible for discrepancies in the results; an additional source of discrepancy in the reported studies might rest in the considerable species differences in myocardial content and metabolism of catecholamines. In addition, it is possible that variations in perfusion and binding under different experimental conditions may account for some of the discrepancies reported for tissue catecholamine content.

J. Hypotheses on the Interaction of Catecholamines and Thyroid States

Although the exact relationship between thyroid hormone and catecholamines has not been fully understood, several attempts have been made to explain nature and mechanism of their interaction.

1. Amount of circulatory catecholamines in the blood

Diller and Kilpatrick (1958) reported that the excretion of A in hyperthyroid patients was increased in parallel with its severity and Goldfien et al. (1961) found an increased A in plasma of hyperthyroid patients. They also reported that in patients with hyperthyroidism, the sensitivity to infused NA was distinctly increased. However, Ishida (1962) reported that both hyper and hypothyroid patients excreted comparable amounts of A, NA and catecholamine metabolites to normal controls, and suggested that the endogenous production of catecholamines was normal in thyroid patients. Urinary levels of NA and metabolites in hyperthyroid patients were not significantly different from the value found in euthyroid subjects (Wiswell et al., 1963).

2. Altered monoamine oxidase (MAO) activity

Novick (1961) has reported that thyroxine treatment causes an increase in heart MAO activity in rats, and Dubnick et al. (1960) have shown the thyroidectomy causes a marked decrease in heart MAO activity of the rat. On the other hand, Spinks (1952) found a decreased MAO activity in the wall of the aorta in thyroxine treated rabbits. Furthermore, Spinks and Burn (1952), Trendelenburg (1953), and Zile and Lardy (1959) found

a decreased MAO activity in the homogenate of liver in rats and rabbits administered pulverized thyroid gland. Spinks and Burn (1952) also reported that MAO activity is increased in the liver homogenate of thyroidectomized rats. Levine et al. (1962) reported that elevated thyroid function was accompanied by decreased tissue levels of MAO, as measured in biopsy specimens of jejunal mucosa of man. Withdrawal of thyroid hormone from the hypothyroid patient was associated with increased tissue MAO activity. Thus, although as Levine et al. (1962) have suggested, altered amine levels induced by alterations in the metabolic enzymes could conceivably contribute to some of the manifestations of human thyroid dysfunction, Ishida (1962) has presented evidence that activities of MAO and O-methyl transferase are normal in thyroid diseases.

3. Decreased contents of catecholamines in organs

According to the classic experiments of Brewster et al. (1956) total epidural block, which abolishes release of A and NA, reduced the elevated oxygen consumptions, heart rates, arterial pressures, and ventricular stroke volume of thyrotoxic dogs to the levels observed in euthyroid animals, but infusion of A and NA in the blocked, thyrotoxic animals resulted in exaggerated hemodynamic and metabolic responses compared to the euthyroid controls. Burn (1958) showed that the spiral strips of rabbit's aorta, in which the tissue catecholamine was previously depleted by reserpine, responded more vigorously than untreated controls. Therefore, he proposed that response of an organ to A or NA was increased with the decrease in the content of catecholamine in that organ. Thus, the exaggerated sensitivity in hyperthyroidism could be easily explained

if the catecholamine content in the cardiovascular system was decreased. This, however, has not been shown but Wurtman et al. (1963) have reported that a part of the mechanism of the increased cardiovascular sensitivity to A found in hyperthyroidism in rats is related to a decreased ability of heart to inactivate catecholamine by binding, and thus leaving more "free" A available to act on its physiologic receptors.

4. Participation of the adenyl cyclase-adenosine-3',5'-phosphate (cyclic AMP) system or phosphorylase *a* in the tissue

Recently the importance of cyclic AMP as a second messenger for hormones has been noted. A number of hormones produce many of their effects by stimulating adenyl cyclase and thereby increasing the level of cyclic AMP within the cells of their target tissues. The concentration of cyclic AMP also depends on the activity of phosphodiesterase for its inactivation (Robison et al., 1970). Cyclic AMP is the chemical trigger mediating catecholamine action in sympathetic target organs (Sutherland and Rall, 1960). There is evidence for cyclic AMP participation in the effect of A and other catecholamines on the force of myocardial contraction (Sutherland et al., 1966). The increased intrinsic heart rate and exaggerated response to sympathetic stimulation in hyperthyroidism can be explained by an increase in tissue cyclic AMP (Hill and Turner, 1968). Thyroid hormone is capable of activating myocardial adenyl cyclase *in vitro*, but this effect is not mediated by the beta adrenergic receptor (Levey et al., 1969). The involvement of cyclic AMP in thyroid function has been recently indicated by studies with thyroid hormones. Thyroid hormone increases the formation of adenyl cyclase and catecholamines have

an activating effect on the enzyme so that either of them, or both, can cause an increase in the amount of cyclic AMP. In contrast, thyroidectomy reduces the amount of the enzyme (Brodie et al., 1966).

The thyroid status alters the lipolytic response by an alteration in the mechanism, presumably involving cyclic AMP, by which the lipase is activated (Fisher and Ball, 1967). Triiodothyronine has been shown to be a competitive inhibitor of cyclic AMP phosphodiesterase, suggesting it exerts its lipolytic action by preventing the degradation of cyclic AMP (Mandel and Kuehl, 1967). Thyroxine appears to induce the formation of additional adenyl cyclase in adipose tissue; in contrast, thyroidectomy causes a reduction in the amount of the enzyme (Krishna et al., 1968). Furthermore, triiodothyronine increases the activity of adenyl cyclase, ultimately stimulating lipolysis with subsequent modulation of mitochondrial regulation by FFA (Challoner, 1969). Several of these investigators have postulated that thyroid hormone may produce their action in adipose and possibly other tissues by decreasing phosphodiesterase. Due to an absence of adequate data, this hypothesis appears to be most applicable to adipose tissue at the present time (Lindsay, 1970).

In rats, the administration of thyroxine, or triiodothyronine causes an increase in cardiac phosphorylase *a* activity which is concomitant with an elevation in mean arterial blood pressure and an increase in heart rate. However, the cardiovascular stimulation and increase in phosphorylase activity observed after intravenous injection of A are not potentiated by prior intramuscular administration of thyroxine (Hess and Shanfeld, 1965). On the other hand, Hornbrook et al. (1965) reported that the administration of thyroxine to rats increases cardiac phosphorylase *a*

levels and potentiated catecholamine response. Similarly, triiodothyronine pretreatment potentiates the effect of NA on phosphorylase a, and triiodothyronine potentiation is not the result of blockade of amine uptake (McNeill and Brody, 1968). However, recent study showed that A causes an increase in cyclic AMP, activation of phosphorylase b kinase and increased activity of phosphorylase a. These metabolic effects of the catecholamine are blocked by the beta adrenergic blocking drug, MJ-1999. Although thyroxine increases myocardial phosphorylase a activity, there is neither an increase in cardiac cyclic AMP nor activation of phosphorylase b kinase. Thus, cardiac phosphorylase a activity is elevated by thyroxine through a mechanism different from that of A (Frazer et al., 1969). These workers suggest that this action may be due to an influence on calcium ions which can elevate phosphorylase a activity without an influence on the cyclic AMP.

5. Altered sensitivity of adrenergic receptors

Thyroid hormones and the catecholamines have been shown to share certain physiologic effects. These hormones interact synergistically, so that the effects of the catecholamines are exaggerated by thyroid hormone excess and attenuated by thyroid hormone deficiency. General adrenergic blockade, in part or in total, reduces the hemodynamic, neuromuscular, and metabolic effects of thyroid hormone excess, without entirely abolishing them. Differential blockade of alpha and beta adrenergic receptors appears to have relatively less effect upon hyperthyroidism, suggesting that both types of receptors are concerned in manifestation of thyroid hormone excess (Waldstein, 1966). Thyroid hormone produces the necessary

intracellular environment without which the steady state and emergency actions of catecholamines would be vitiated. In hyperthyroidism the increased concentration of thyroid hormones results in a lowering of the threshold for catecholamine action. For this reason it is possible to alleviate many of the symptoms of thyrotoxicosis by means of drugs which block beta adrenergic receptors (Parsons and Ramsay, 1968). However, it is necessary to be cautious on the interpretation of thyroid hormone potentiation of catecholamine actions *in vitro*. The potentiation by thyroid hormones of the A- or NA-induced contraction of rabbit helical aortic strips was caused by chelation of contaminating copper in the Krebs-Ringer medium (Shida *et al*., 1963) by the thyroxine, analogous to the removal of copper by EDTA.

K. The Purpose of the Present Study

The hypothesis to be tested in this study is that the levels of thyroid activity is related to altered activity in adrenergic nerves in contrast to changes in sensitivity of the responding organs. Specifically, this study is undertaken to determine if, in various thyroid states, there are differences in the amount, uptake and release of NA in tissues with high adrenergic innervation.

In the past, experimental hyperthyroidism in animals has been induced by the administration of large doses of thyroid hormone for a short duration, such as 7-10 days. Such treatment may cause a functional change to occur, but it is uncertain whether histological changes typical of hyperthyroidism are induced. Since both hyperthyroidism and hypothyroidism in

man are usually chronic, the administration of a small dose of thyroid hormone for longer periods, perhaps a few months, may be more reasonable as a method of inducing experimental hyperthyroidism in animals. It is well known that differences in the duration of the administration of a drug may account for some of the different results in drug action (Schildkraut et al., 1971; Neff and Costa, 1967; Javoy et al., 1968; Schubert et al., 1970), which may also be true in this case.

In the present study five tissues of rats, namely, the heart, brain, salivary gland, adrenal gland and vas deferens were used to investigate whether changes in catecholamine turnover were possible under chronic hyper and hypothyroidism, and if so, whether it is general in all the tissues.

MATERIALS AND METHODS

A. Animals

Young-adult Sprague-Dawley, male rats weighing from 125 to 150 g were purchased from Hormone Assay Laboratories Inc., Chicago. Some of the animals were thyroidectomized and some sham-operated by the Hormone Assay Laboratories 4 days prior to delivery to this laboratory. All rats were kept, 4-5 to a cage, in a small, well ventilated, temperature controlled room at 22-23°C. The room was kept locked at all times to avoid disturbances by other than the investigator and necessary visits by animal attendants for feeding and cleaning. To control the influence of environmental lighting on growth in the rat (Fiske, 1941) the room lighting was controlled with lights on for 12 hours (7:00 A.M. to 7:00 P.M.) and off for 12 hours each day. Other sources of light were minimal.

Diet and water supply for the animals varied somewhat depending upon the conditions of the experiment. In the preliminary experiments, normal animals which were not thyroidectomized, were fed on the normal vivarian rat diet, 'Purina Laboratory Lab Chow' and the water supply was normal city 'tap water'. For experiments involving thyroidectomized animals a special low iodine diet, "Remington Type, Nutritional Biochemical Co.", was used and the water supply was prepared with a specific iodine content due to added potassium iodide.

B. Drugs and Reagents

1. Aluminium oxide (Chromatographic adsorption analysis grade), The British Drug Houses Ltd., Poole, England.

2. Adrenaline - l-Epinephrine bitartrate, Sterling-Winthrop Research Institute. Rensselaer, N.Y., U.S.A.

3. Noradrenaline - l-norepinephrine bitartrate, Winthrop Laboratories, New York, N.Y., U.S.A.

4. Tritiated noradrenaline - dl-Norepinephrine-7-^{3}H, New England Nuclear Corporation, Boston, Mass., U.S.A.

5. Dopamine (3,4-dihydroxyphenylethylamine) hydrochloride, Nutritional Biochemical Company, Cleveland, Ohio, U.S.A.

6. l-Thyroxine, sodium salt, pentahydrate ($C_{15}H_{10}I_4NNaO_4.5H_2O$), m.p. 245-246°C, Aldrich Chemical Co., Inc., Milwaukee, Wis., U.S.A.

The net thyroxine base used for the dose was calculated from the pentahydrate, sodium salt of l-thyroxine as follows:

Sodium thyroxine x 0.874 = net thyroxine.

$$\frac{C_{15}H_{11}I_4NO_4}{C_{15}H_{10}I_4NNaO_4.5H_2O} = \frac{776.93}{888.95} = 0.874$$

Thyroxine solution

Thyroxine was dissolved with one volume of 0.1N NaOH, then diluted with 9 volumes of 1% NaCl; thus thyroxine was dissolved in 0.01N NaOH-0.9% NaCl solution, subsequently referred to as alkaline saline solution.

A fresh thyroxine solution was made every 3-4 days to prevent a possible deterioration. When the solution was not in use it was stored in a refrigerator.

7. Ether: Anesthesia grade, U.S.P. Fisher Scientific Company, Fair Lawn, N.J., U.S.A.

C. Induction of Hyper and Hypothyroid States and Control Animals

Hyperthyroidism in the rats was induced by daily subcutaneous injection of l-thyroxine. The amounts of thyroxine and the duration of administration depended on the experiments performed and are summarized in Table I. Hypothyroid and control animals were given daily subcutaneous injections of 1 ml/kg of alkaline saline solution, which is the same volume of solution as that used for treating the hyperthyroid groups.

In the preliminary experiments A_1 and A_2, hyperthyroidism was induced by administering thyroxine to normal rats (without thyroidectomy). Paired, age-matched control rats, and hyperthyroid rats were fed with a normal fortified laboratory diet, and all rats, in eu-, hyper- and hypothyroid groups, were allowed free access to drinking water. To avoid differences in the diet and to try to keep all animals under the same conditions, in Experiments I and II hyperthyroidism was induced by administering thyroxine to thyroidectomized rats. For these experiments normal control rats were sham-operated, and all rats in eu-, hyper- and hypothyroid groups were fed on the low iodine diet and were allowed free access to drinking water containing 37.5 (Experiment II) - 75 (Experiment I) μg

TABLE I

RATS	EXPT.	THYROXINE (μg/kg/day)	DURATION OF ADMINISTRATION	DIET
With thyroid gland (non-thyroid-ectomized)	A_1	3	7 days	Normal
		25	11 "	"
		50	5 "	"
	A_2	100	8 weeks	"
		200	7 "	"
		400	3 "	"
		800	2 "	"
Without thyroid gland (thyroid-ectomized)	I	100	4 "	Low iodine
		25	4 "	"
	II	25	12 "	"

per cent iodine in the form of potassium iodide.

Hypothyroidism was induced by thyroidectomy and the animals were maintained on the low iodine diet. In the preliminary experiments A_1 and A_2, the water supply was normal tap water but in Experiments I and II it was iodine controlled water, as described above. Paired, age-matched controls for the experiments using thyroidectomized animals (Experiment I and II) were sham-operated.

Euthyroid rats, with the thyroid glands intact and fed with a low iodide diet can utilize iodide in the drinking water. Hyperthyroid rats without the thyroid glands cannot utilize iodine in the drinking water, but become hyperthyroid due to the administration of thyroxine. Hypothyroid rats without the thyroid glands cannot utilize iodide in the drinking water and develop the hypothyroid state. The conditions which existed in Experiments I and II are summarized in Table II.

D. Assessment of Thyroid State

The level of thyroid activity was determined by the changes of the body weight and heart rate. In preliminary experiments the hyperthyroidism was also confirmed by measuring the oxygen consumption and rectal temperature.

1. Body weight measurements

The rats were weighed on an animal scale (sensitivity 0.1g), between 9:00 A.M. and 12:00 Noon. In Experiment A_1 the rats were weighed daily but for Experiment A_2, I and II the rats were weighed before and

TABLE II

CONDITIONS OF THE ANIMALS IN EXPERIMENTS I AND II

Thyroid State	Operation	Thyroid	Low Iodine Diet	Iodine in Water	Injections: Thyroxine Solution	Injections: Alkaline Saline
Euthyroid	Sham-operated	+	+	+	-	+
Hyperthyroid	Thyroidectomized	-	+	+	+	-
Hypothyroid	Thyroidectomized	-	+	+	-	+

after the start of the experiment, and also immediately before sacrificing.

2. Heart rate measurements

The heart rates of all animals were determined before and after the start of the experiment, and also one day before the animals were killed. In most experiments the heart rates were measured in ether anesthesized animals by taking electrocardiograms on a Beckman Dynograph recorder, (Taylor *et al*., 1967). Three needle electrodes were placed into subcutaneous tissues of the anesthesized animals, two in the upper fore limbs and one on the midline of the backs near the tail. In the preliminary experiments for measuring oxygen consumption and rectal temperature, some of the heart rate measurements were made under barbiturate and some under ether anesthesia but, as ether anesthesia was found to be more easily controlled and reliable, it was used in all subsequent experiments for determination of heart rates.

The heart rates of hyperthyroid animals were significantly higher and the heart rate of hypothyroid rats were significantly lower than those of euthyroid animals. During the treatment period, hypothyroid animals which had normal heart rates were eliminated from their group as these animals may not have been satisfactorily thyroidectomized.

3. Oxygen consumption and rectal temperature measurements

Oxygen consumption measurements were made in an apparatus which was a modification of that described by Guyton and Farish (1959). Fig. 1 is a diagram of the apparatus which functions on the basis of the changes of oxygen content in air flowing through the animal chamber. The temper-

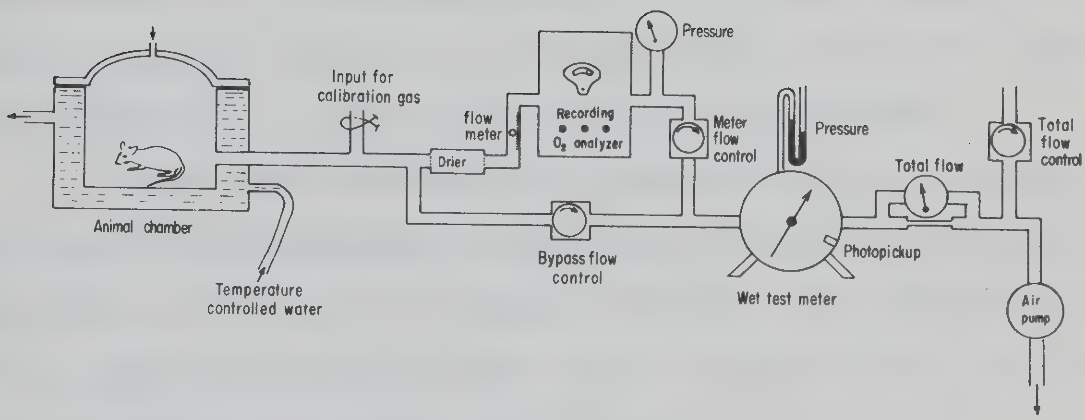


Fig. 1. Diagram of the apparatus for the measurement of oxygen consumption.

ature of the chamber was maintained at 24°C and the oxygen in the air flowing through the chamber was measured before and during the time the animal was in the chamber. Changes observed were due to the oxygen utilized by the animal. This method was used for both anesthesized and unanesthesized animals and the rectal temperature measurements and electrocardiograph recordings were made simultaneously with the oxygen consumption tests on anesthesized rats. In the unanesthesized rats the oxygen consumption measurements were made separately from the temperature and heart rate measurements as the latter required that the animals be anesthesized.

Rectal temperatures were first measured with a Yellow Springs Instrument Co. Telethermometer and later with a thermocouple probe with recording on an Electronic 19, Honeywell Recorder. After anesthesizing the rat, the probe was placed in the rectum, 5 cm from the anus, and the rectal temperature was recorded for one minute.

E. Radioactive Tracer Techniques

dl-Noradrenaline-7-^{3}H (specific activity: 39.1 μc/μg NA in Experiment A_2; 51.8 μc/μg NA in Experiment I; 58.8 μc/μg NA in Experiment II) was diluted to 40 μc/ml with acidified isotonic saline, pH 2. Tritiated NA was administered at a dose of 100 μc/kg to unanesthesized rats via the tail vein. The animals were decapitated by a guillotine, 3 and 27 hours after injection in Experiment A_2 and 15 minutes, 3, 6, 12 and 24 hours after injection in Experiment I and II. The heart, brain, salivary glands, adrenal glands (in Experiment A_2, I and II) plus vasa defferentia (in Experiment I and II) were immediately removed, frozen in liquid nitrogen and

stored in a cold room at -20°C until catecholamine determinations and counting were done later on the same day and the following day. Radioactivity counting was done in a Nuclear Chicago scintillation counter using 0.1 ml samples of the alumina eluate in 10 mls of a scintillation fluor containing the following ingredients:

PPO (2,5-diphenyloxazole)	15.2 g
POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene]	0.38 g
Solubilizer (BBS-3)	500 ml
Toluene	3,285 ml

Efficiency of counting was estimated by the channels ratio method and radioactivity was expressed as DPM/g tissue.

F. Catecholamine Assay Techniques

1. Extraction of catecholamines from the tissues

The frozen tissue was weighed, crushed and homogenized in 4 volumes of cold 0.4N perchloric acid ($HClO_4$) with a glass Potter homogenizer. The homogenate was centrifuged at 3000 rpm for 5 minutes in an International Centrifuge (Universal model, UV) and 2 ml of clear supernatant fluid was transferred to a small beaker containing 500 mg of activated aluminium oxide (Al_2O_3) and 7 mls of 0.5M Tris buffer. The mixture was stirred on a magnetic stirrer and the pH adjusted to 8.4 with the aid of a pH meter. The mixture was then transferred to a stoppered centrifuge tube and gently shaken on a Teckni Lab Instrument Co. Rotator, Model 71, for 15 minutes. After shaking the mixture was centrifuged at 3000 rpm for 5 minutes and the supernatant fluid was aspirated off and discarded. The alumina dep-

osit was resuspended and washed with 10 mls of distilled water before again centrifuging and discarding the supernatant washings. The adsorbed catecholamines were eluted from the alumina by addition of 3 mls of 0.05N $HClO_4$ and gently shaking for 15 minutes prior to centrifugation.

2. Oxidation and fluorescence measurements

To a 1 ml aliquot sample of the clear supernatant eluate there was added, in timed sequence, 1 ml of 0.1M EDTA reagent, 0.2 ml of I_2 oxidizing reagent which was allowed to act for exactly 2 minutes before adding 0.2 ml of alkaline sulphite reducing reagent which was followed in exactly 2 minutes with 0.2 mls of 5N acetic acid reagent. The mixtures were shaken after each addition and, after the addition of the acetic acid, were placed in a boiling water bath for 3 minutes for fluorescence development. The tubes were then cooled rapidly in a cold water bath and allowed to come to room temperature, and the solutions were placed in quartz cuvettes for fluorescence determinations.

The fluorescence of each sample was determined with an Aminco-Bowman spectrophotofluorometer at three different wavelengths and calculations for each amine was done by use of the following equation and the differential equations below.

$$\mu g\ CA/g\ tissue = (FS - FTB)/FSt \times 0.5 \times 3/1 \times 5/2 \times \%\ Recovery,$$

where:

FS = fluorescence of the sample

FTB = fluorescence of the tissue blank

FSt = fluorescence of the standard (0.5 μg/ml)

3/1 and 5/2 = dilution factors.

Wavelengths used in these determinations were:

	Wavelengths (mμ) Activation	Emission
Adrenaline	410	500
Noradrenaline	385	485
Dopamine	320	370

3. Differential calculations

The content of A and NA in the tissues were calculated by the differential fluorescence method reported by von Euler (1959) and values were corrected for a recovery of approximately 65%, determined for each tissue as described below. At the wavelengths indicated above the relative fluorescence of the lutines were sufficiently different to allow their estimations in the equations:

$$A = yNAa + xAa$$

$$B = YNAb + xAb$$

where:

x and y are the amounts of A and NA;

A and B are the fluorescence readings for wavelengths a (for A, 410/500) and b (for NA, 385/485); and

Aa, Ab, NAa, and NAb are the fluorescence readings per 0.5 μg, with wavelength settings a and b for adrenaline and noradrenaline respectively.

For the estimation of DA the same method was used with DA substituted for A in these equations. This would create a small error but tests showed that the estimate for DA was reasonably correct. The endo-

genous DA content was measured in all the tissues tested. However, in the heart, salivary glands and vasa defferentia the DA values obtained were so low they were omitted, and only the values of the brain and adrenal glands were shown. The calculation of this data was programmed for an Olivetti-Underwood Programma (Model 101), and this program was used for all calculations.

4. <u>Determination of the recovery of catecholamines from the tissues</u>

Concurrently with each set of catecholamine determinations separate controls and recovery determinations were made on the tissues of two rats. After homogenizing the tissues as indicated above the homogenate from each tissue was divided equally. To one sample of the homogenate 0.5 μg of NA was added to form internal standard a (ISa) and to the second was added an equal volume of the 0.05N perchloric acid solvent, to form internal standard b (ISb). The extraction and fluorescence developments were done for each tissue as described above and the percentage recovery of the added NA was calculated as follows:

$$\% \text{ Recovery} = [3(\text{ISa} - \text{ISb})/\text{NA Std}] \times 100$$

where:

3 is the dilution factor,

ISa is the fluorescence of the internal standard a,

ISb is the fluorescence of the internal standard b, and

NA Std is the fluorescence of the NA standard solution (0.5 μg/ml of NA).

5. Preparations of blanks, standards and reagent solutions

Tissue blanks were prepared by treating a 1 ml aliquot sample of the alumina eluate as in other oxidation tests except that the orders of adding the alkaline sulphite reagent and the iodine reagent were reversed so that no oxidation took place. The reading of the tissue blank was subtracted from the reading for the test solution.

Reagent blanks were prepared by treating a 1 ml sample of 0.05N $HClO_4$ as in other oxidation tests but as no catecholamine was present no color was developed. This sample was used to adjust the zero reading on the fluorometer.

Standard catecholamine solutions were prepared for adrenaline, noradrenaline and dopamine at a concentration of 0.5 μg/ml in 0.05N $HClO_4$. One ml samples of each of these were oxidized along with each set of tissue tests as reference standards for calculation of the catecholamine content of the tissue samples.

The Tris buffer used was a 0.5M solution of tris(hydroxymethyl) aminomethane base which was titrated to pH 9 with 2N HCl.

The 0.1M EDTA reagent used was prepared by dissolving 37.2 g of disodium ethylenediamine tetraacetate dihydrate in a litre of 1M sodium acetate solution. The pH was adjusted to 7 with 5N NaOH.

The iodine reagent was prepared by dissolving 1.27 g of iodine crystals in 100 ml of absolute alcohol.

The alkaline sulphite reagent was prepared by diluting 1 ml of 25% Na_2SO_3 (anhydrous) in 9 mls of 5N NaOH. This solution was freshly

prepared just prior to use.

6. Statistical method

The standard errors of the means of the results were calculated using the following formula:

$$SE = \sqrt{\frac{\Sigma x^2 - (\Sigma x/n)^2}{n(n-1)}}$$

Student's t-test was used to calculate the significance of the difference between the two different states (such as euthyroid group and hyperthyroid group, or euthyroid group and hypothyroid group) which were compared:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{\Sigma(x - \bar{x})^2 + \Sigma(y - \bar{y})^2}{Nx + Ny - 2}(1/Nx + 1/Ny)}}$$

where:

Nx and Ny are the sample sizes,

$\bar{x} - \bar{y}$ is the difference between the sample means, and

$\Sigma(x - \bar{x})^2$ and $\Sigma(y - \bar{y})^2$ are the sums of squared differences.

P values were obtained from Fisher's distribution tables.

When an exceptionally large or small value was found in the samples of values obtained in the experiments, Grubbs' test (1950) was used to help make a decision on whether such a value may be discarded:

$$\bar{x} = \Sigma x/n \qquad S^2 = \Sigma(x - \bar{x})^2/n$$

$$S = \sqrt{\Sigma(x - \bar{x})^2/n} \qquad T_n = |x_n - \bar{x}|/S$$

where:

$\bar{x}$ is sample mean,

n is sample size,

$\Sigma(x - \bar{x})^2$ is sum of squared differences,

S is the standard deviation,

x_n is an exceptionally large or small value, and

T_n is a value compared with the value obtained from Grubbs' T_n table. If the values of T_n obtained through calculation exceeds the value given in the following table, the questionable value can be discarded:

Frank E. Grubbs

N	1%	2.5%	5%	10%
3	1.414	1.414	1.412	1.406
4	1.723	1.710	1.689	1.645
5	1.955	1.917	1.869	1.791
6	2.130	2.067	1.996	1.894
7	2.265	2.182	2.093	1.974
8	2.374	2.273	2.172	2.041
9	2.464	2.349	2.237	2.097
10	2.540	2.414	2.294	2.146
11	2.606	2.470	2.343	2.190
12	2.663	2.519	2.387	2.229
13	2.714	2.562	2.426	2.264
14	2.759	2.602	2.461	2.297
15	2.800	2.638	2.493	2.326
16	2.837	2.670	2.523	2.354
17	2.871	2.701	2.551	2.380
18	2.903	2.728	2.577	2.404
19	2.932	2.754	2.600	2.426
20	2.959	2.778	2.623	2.447
21	2.984	2.801	2.644	2.467
22	3.008	2.823	2.664	2.486
23	3.030	2.843	2.683	2.504
24	3.051	2.862	2.701	2.520
25	3.071	2.880	2.717	2.537

RESULTS

A. Preliminary Experiments

1. Evaluation of the heart rate measurement method of determining the thyroid state

The heart rate, oxygen consumption and rectal temperature were measured simultaneously in the same animal, to evaluate the heart rate method of determining changes in thyroid states. Under anesthesia due to intraperitoneal administration of 50 mg/kg of sodium pentobarbital or of 250 mg/kg of sodium barbital, the heart rate was about 360 beats/min, the oxygen consumption 0.013-0.018 ml/min/g body weight, and the rectal temperature approximately 36°C, when these were measured simultaneously in normal rats (Table III). When these physiological properties were measured separately with different experimental conditions the values obtained varied somewhat from the above. The heart rate under ether anesthesia, was approximately 400 beats/min, the oxygen consumption, without anesthesia, was 0.016-0.017 ml/min/g body weight, and the rectal temperature under anesthesia due to sodium barbital (200 mg/kg) was 38°C. In hyperthyroid animals the heart rate, oxygen consumption and rectal temperature were 510-550 beats/min, 0.023-0.024 ml/min/g, and 39°C respectively, which were significantly increased when compared with the control animal (Fig. 2). These results are consistent with those reported by Leblond and Hoff (1944) who said that thyroxine increases, in a parallel fashion, the heart rate, oxygen consumption and body temperature of rats. Thus the heart rate can be used as an indicator of changes in the thyroid state in these experiments.

TABLE III

HEART RATE, OXYGEN CONSUMPTION AND RECTAL TEMPERATURE IN EUTHYROID RATS

Group	No. of Animal	Body Wt (g)	Heart Rate (beats/min)	O_2 Consumption (ml/min/g)	Rectal Temperature(°C)
1	10	370±12.6*	360±11	0.018±0.0011	35.9±0.2
2	8	354±11.5	358± 9	0.013±0.0008	36.2±0.3

* Mean±S.E.

Temperature of Chamber = 24°C.

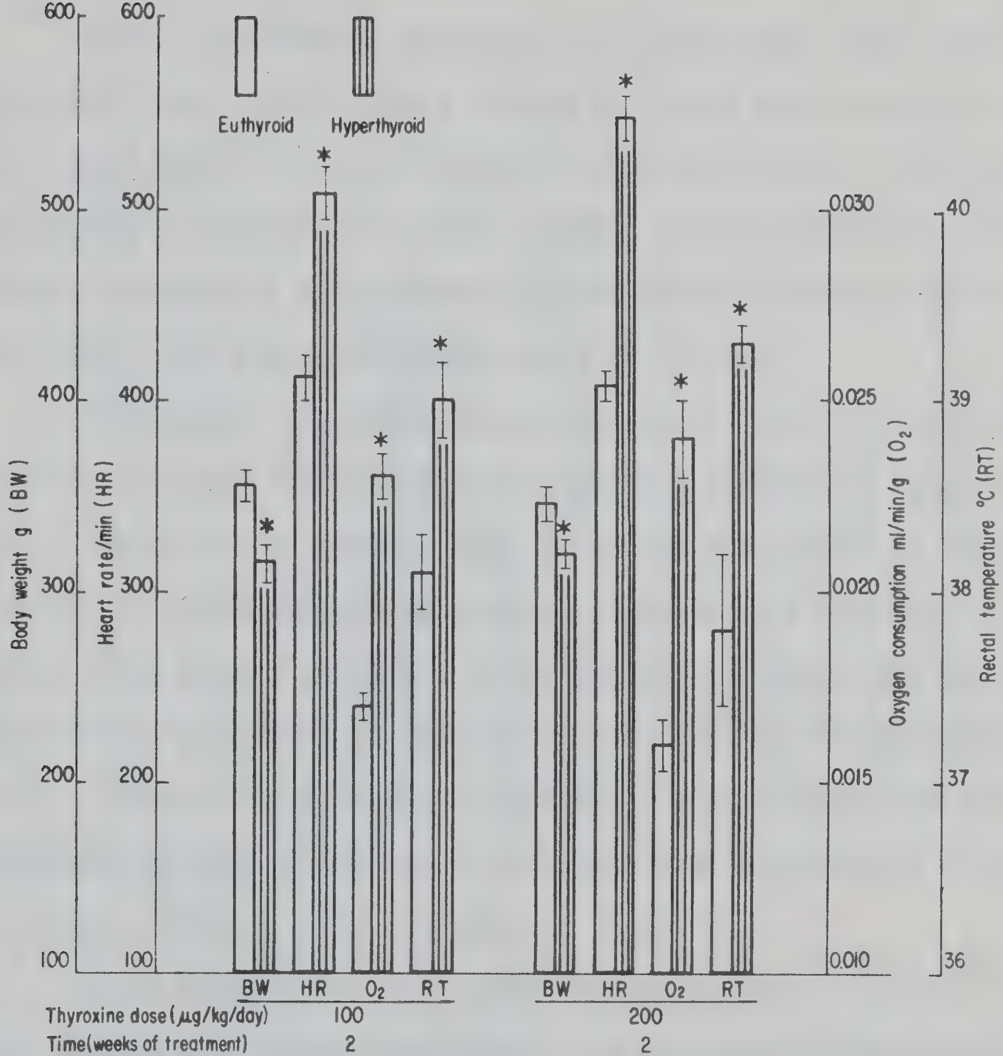


Fig. 2. The influence of the dose of thyroxine on the body weights, heart rates, oxygen consumption and rectal temperature of rats. Age-matched normal rats were treated with 100 or 200 µg/kg/day of thyroxine for two weeks. The euthyroid control animals received 1 ml/kg/day of alkaline saline solution. I = S,E. of the mean; * indicates $p<0.05$, treated vs euthyroid controls. No. of animals: 5-8/group. Data for this figure are tabulated in Table X of the Appendix.

2. Determination of an adequate dose of thyroxine --(Experiments A_1, A_2 and I)

In most experiments reported in the literature, large doses of thyroxine have been used for short periods to induce hyperthyroidism in animals. Such doses are fatal if used for prolonged periods. In this work we wished to simulate the chronic disease state and therefore some preliminary experiments were necessary to determine an adequate dose of thyroxine which could be administered for 8 to 12 weeks.

Twenty-eight thyroidectomized rats and 37 normal rats were divided into three groups for hypothyroid, thyroxine treated and euthyroid controls. Thyroxine was given to half of the normal animals in increasing doses of 3, 25 and 50 μg/kg/day for 7, 11 and 5 days respectively. At the end of seven days of treatment at 3 μg/kg/day the heart rate was not different from the controls so the dose was increased to 25 μg/kg/day of thyroxine. However, at the end of a further 11 days of treatment the heart rate elevation was not significantly different from the controls so the dose was again elevated to 50 μg/kg/day of thyroxine.

In the hypothyroid group (thyroidectomized rats) the heart rate and body weights were markedly different from the control animals as indicated in Fig. 3. These thyroidectomized animals did not gain weight throughout the experiment and their heart rates continued to fall relative to the euthyroid controls.

The doses of thyroxine used in Experiment A_1 did not appear likely to induce a satisfactory hyperthyroid condition even if given for prolonged periods. Therefore a second experiment (A_2) was done using a series of

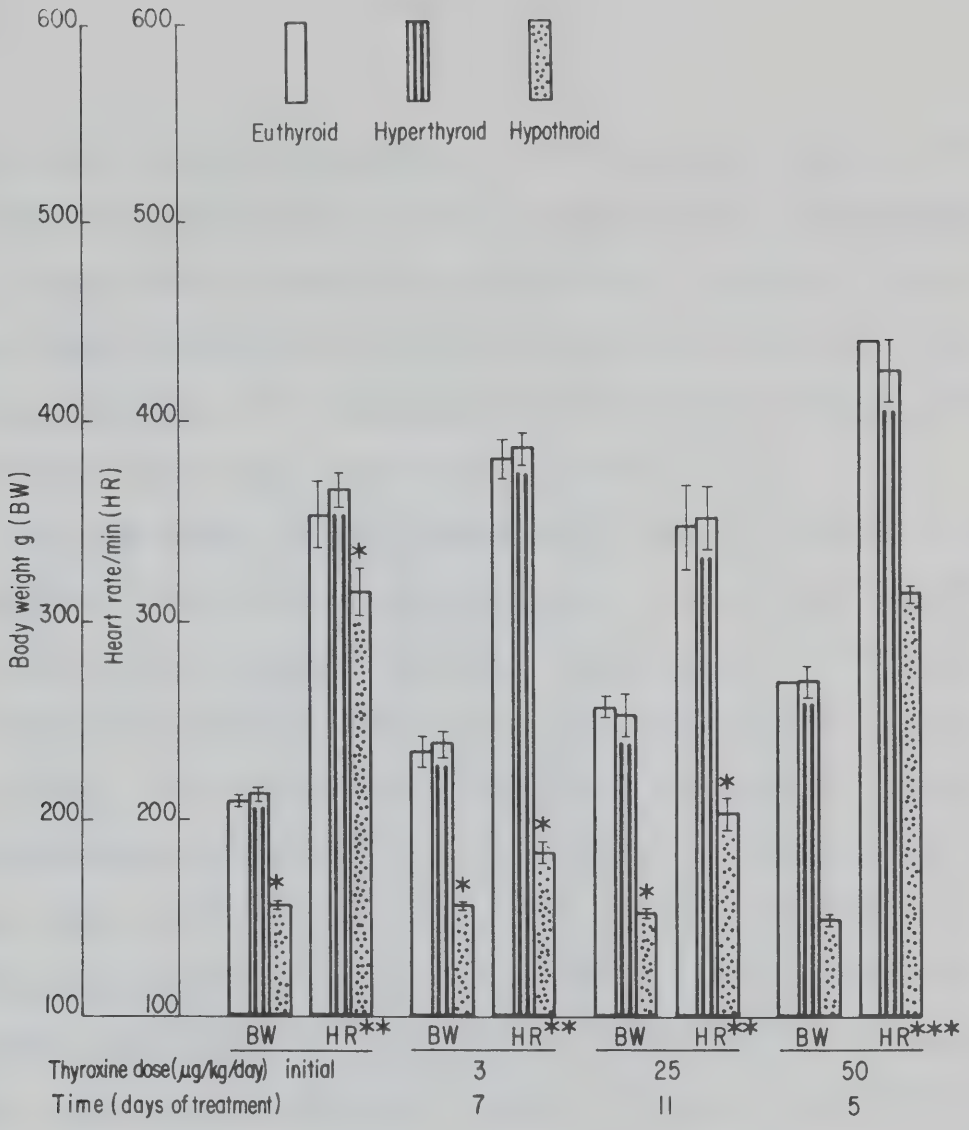


Fig. 3. The influence of the dose of thyroxine and the duration of treatment on the body weights and heart rates in rats (Experiment A_1). Age-matched normal rats were treated with 3 µg/kg/day of thyroxine for the first 7 days and 25 µg/kg/day for 11 days followed by 50 µg/kg/day for 5 days. The hypothyroid rats were thyroidectomized and they, as well as the control rats, received 1 ml/kg/day of alkaline saline solution. I = S.E. of the mean; * indicates $p<0.05$, treated vs euthyroid controls; ** indicates sodium barbital anesthesia (250 mg/kg/i.p.); *** indicates ether anesthesia. Data for this figure are tabulated in Table XI and XII of the Appendix.

higher doses for varying time periods. In this experiment doses of 100, 200, 400 and 800 µg/kg/day of thyroxine were administered subcutaneously for 8, 7, 3 and 2 weeks respectively to normal rats. A similar group of normal rats were maintained as controls and were also treated daily by subcutaneous injections of 1 ml/kg/day of alkaline saline solution which was equivalent to the volume given to the thyroxine groups.

One week after starting treatment with 100-800 µg/kg/day of thyroxine the heart rates of all of the animals were significantly increased in comparison with the euthyroid controls. Tables IV and V respectively show the influence of various doses on the heart rates and body weights of these animals. The animals given larger doses were used for catecholamine determinations at the ends of their respective treatment periods of one or two weeks, so did not live long enough to show toxic effects of prolonged administration. However, it was observed that the death rate among animals given 200 or more µg/kg/day was elevated after 2 weeks of treatment, indicating that these dose levels, and possibly the 100 µg/kg/day, were too high to simulate chronic hyperthyroidism.

In Experiment I the 100 µg/kg/day dose level was used, but those animals were thyroidectomized and the number of deaths at that dose level was again too high. For that reason the dose of 25 µg/kg/day was tried with thyroidectomized rats and found to maintain a satisfactory elevation of heart rate, with a low mortality rate. As indicated in Fig. 4, in the course of the 8 weeks of Experiment I the body weight of the hyperthyroid animals at first increased faster than the controls, while the animals were young, but gradually this difference disappeared and their weights became significantly less than the control animals at the end of 8 weeks. Therefore the dose level of 25 µg/kg/day was considered adequate for in-

TABLE IV

THE INFLUENCE OF THYROXINE DOSE AND DURATION OF TREATMENT ON THE HEART RATES OF RATS

Thyroid State	Thyroxine Dose (μg/kg/day)	Weeks of Treatment					
		0	1	2	4	7	8
Euthyroid		443±13 (8)*	379±14 (8)	392±11 (8)	413±12 (8)	-	402±5 (8)
Hyperthyroid	100	456±10 (8)	487±12 (8)†	485±11 (8)†	508±14 (8)†	-	507±9 (8)†
Euthyroid		435±14 (8)	420±11 (8)	404±10 (8)	408± 8 (5)	398±5 (5)	
Hyperthyroid	200	456± 6 (8)	510±15 (8)†	511±13 (7)†	549±12 (6)†	489±7 (6)†	
Euthyroid		445±10 (8)	410±13 (7)	386±11 (7)			
Hyperthyroid	400	434± 9 (8)	551±20 (7)†	521±27 (7)†			
Euthyroid		435±11 (8)	389± 7 (8)	393± 9 (8)			
Hyperthyroid	800	446±15 (8)	563±13 (8)†	556±11 (8)†			

* Mean±S.E. (beats/min).

† $p<0.05$.

TABLE V

THE INFLUENCE OF THYROXINE DOSE AND DURATION OF TREATMENT ON THE BODY WEIGHTS OF RATS

Thyroid State	Thyroxine Dose (μg/kg/day)	Weeks of Treatment 0	1	2	4	7	8
Euthyroid		192± 3 (8)*	266± 4 (8)	295± 5 (8)	356± 8 (8)	-	432±7 (8)
Hyperthyroid	100	187± 3 (8)	244± 4 (8)†	270± 3 (8)†	315± 9 (8)†	-	382±7 (8)†
Euthyroid		178± 7 (8)	234±14 (8)	281±14 (8)	348±15 (5)	405±9 (5)	
Hyperthyroid	200	180± 2 (8)	231± 4 (8)	271± 5 (7)	320± 8 (6)	368±5 (6)†	
Euthyroid		168±12 (8)	242± 9 (7)	292± 8 (7)			
Hyperthyroid	400	184± 3 (8)	225± 9 (7)†	264± 8 (7)†			
Euthyroid		184± 2 (8)	246± 4 (8)	270± 4 (8)			
Hyperthyroid	800	184± 2 (8)	218± 4 (8)†	241± 6 (8)†			

* Mean±S.E. (weight in g).

† $p<0.05$.

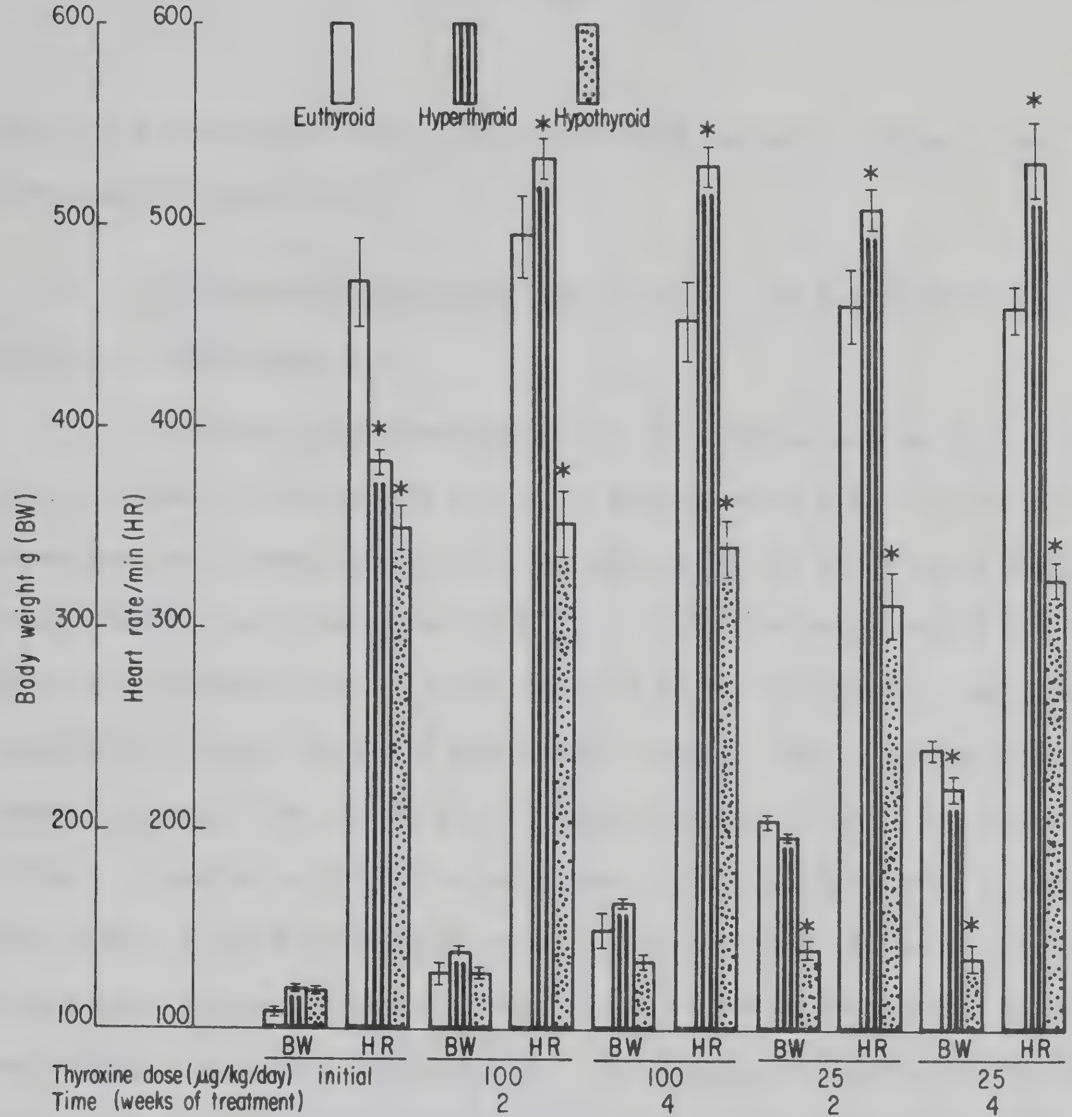


Fig. 4. The influence of the dose of thyroxine and the duration of treatment on the body weights and heart rates of rats (Experiment I). Three groups of age-matched rats were used for hyper, hypo and euthyroid controls. The hyperthyroid group were thyroidectomized and treated with 100 µg/kg/day of thyroxine for 4 weeks, after which the dose was reduced to 25 µg/kg/day for the remaining 4 weeks. The hypothyroid group were thyroidectomized and treated with 1 ml/kg/day of alkaline saline solution. The euthyroid control group were sham-operated but were treated daily with the alkaline saline injection comparable to the other groups. I = S.E. of the mean; * indicates $p<0.05$, treated vs euthyroid controls. No. of animals: 5-9/group. Data for this figure are tabulated in Tables XIII and XIV of the Appendix.

ducing a hyperthyroid state, for a prolonged period. That dose was therefore used in Experiment II.

3. Catecholamine determinations in normal and thyroxine treated animals -- Experiment A_2

Data for the determinations of the catecholamines in the tissues of the animals of Experiment A_2, which were treated with high doses of thyroxine for a short period (400 and 800 µg/kg/day for 3 and 2 weeks respectively) are illustrated in Fig. 5. With the exception of the adrenal gland the A content was very low relative to the NA content, and DA was measurable in only the brain and adrenal glands. Fig. 5 shows that in the adrenal glands A, NA and DA are all significantly lower in the hyperthyroid animals as was DA in the brain but A was significantly higher in the salivary gland of the hyperthyroid animals. In the other tissues, also, the mean values showed a tendency toward less catecholamines in the hyperthyroid animals on a µg/g tissue basis. The tissue weight changes shown in Table XV in the Appendix indicate a slight hyperplasia of some peripheral tissues in hyperthyroid animals which may account for this trend. The increase in tissue weight was significant in the hearts and adrenal glands only.

Fig. 6 indicates the catecholamine content of the tissues of the animals treated at a lower dose for a longer period (100 and 200 µg/kg/day of thyroxine for 8 and 7 weeks respectively). These data indicated significant differences between the euthyroid and hyperthyroid groups with respect to the A in the heart, the NA in the brain and the NA and DA in the adrenal glands. These differences were all in the direction of a reduction

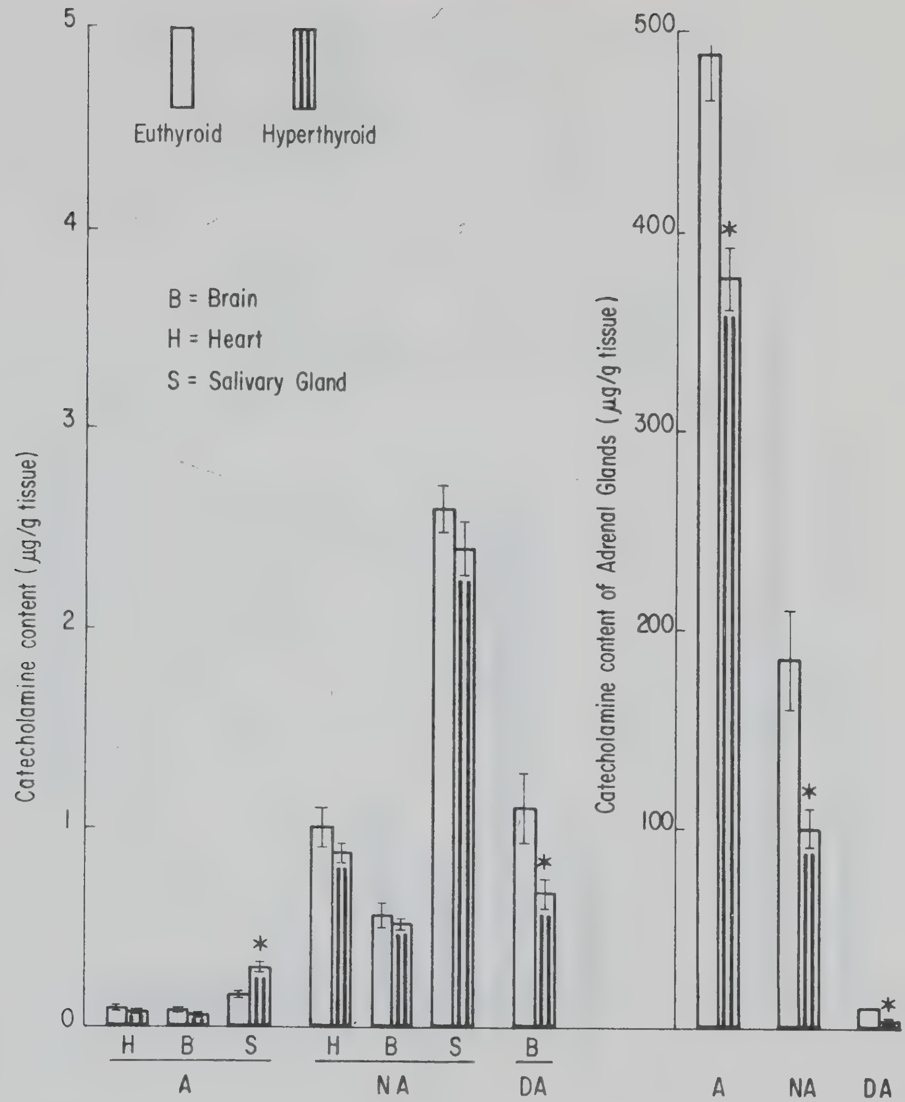


Fig. 5. The influence of the dose of thyroxine and the duration of treatment on A, NA and DA contents in rat tissues (Experiment A_2). Age-matched normal rats were treated with 700-800 µg/kg/day of thyroxine for 3-2 weeks, with the lower dose given for longer periods. The control rats received 1 ml/kg/day of alkaline saline solution. I = S.E. of the mean; * indicates $p<0.05$, treated vs euthyroid controls. No. of animals: 15/group for A and NA measurements and 1-12/group for DA measurement. Data for this figure are tabulated in Table XV of the Appendix.

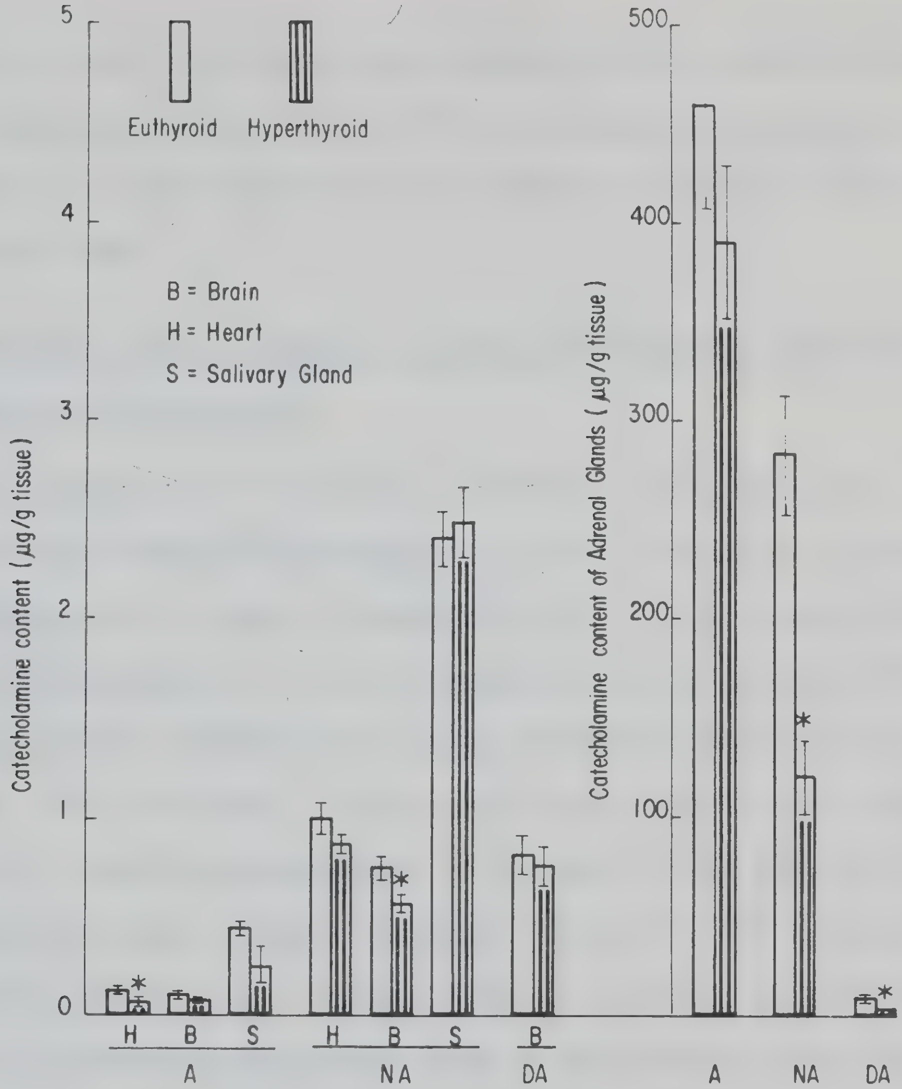


Fig. 6. The influence of the dose of thyroxine and the duration of treatment on A, NA and DA contents in rat tissues (Experiment A_2). Age-matched normal rats were treated with 100-200 μg/kg/day of thyroxine for 8-7 weeks, with the lower dose given for longer periods. The control rats received 1 ml/kg/day of alkaline saline solution. I = S.E. of the mean; * indicates $p<0.05$, treated vs euthyroid controls. No. of animals: 13-14/group for A and NA measurements, and 4-12/group for DA measurement. Data for this figure are tabulated in Table XVI of the Appendix.

of the µg CA/g tissue in the hyperthyroid animals but, as indicated above, the tissue weights tabulated in Table XVI in the Appendix, indicate a slight hyperplasia in the tissues from the hyperthyroid group, which may account for this trend.

4. <u>Uptake and loss of ^{3}H-NA by tissues of normal and thyroxine treated animals -- Experiment A_2</u>

The retention of intravenously injected ^{3}H-NA by the hearts of euthyroid rats and those made hyperthyroid by administration of thyroxine, 100-200 µg/kg/day for 7-8 weeks is compared in Fig. 7. Three hours after intravenous administration of ^{3}H-NA the concentration in the heart ($259 \pm 12 \times 10^3$ DPM/g) of the hyperthyroid rats was lower than that of normal controls ($302 \pm 40 \times 10^3$ DPM/g). On the other hand 27 hours after the administration of ^{3}H-NA the concentration in the heart of the hyperthyroid rats was higher than that of normal controls. After 27 hours the cardiac ^{3}H-NA of hyperthyroid rats was reduced to 35% of the amount at three hours after administration while the cardiac ^{3}H-NA of the normal controls was 21% of the amount at three hours after the injection. When the tissue content of the heart was plotted against time, Fig.7, the degradation curve of the hyperthyroid rats was less steep when compared with that of the normal control, showing a slower fall in the radioactivity in the heart of the hyperthyroid animals. Table VI shows the radioactivity of the various tissues at 3 and 27 hours after injection of ^{3}H-NA. No significant change was observed between the hyperthyroid and normal control animals with regard to the brain and salivary glands but, in contrast to the heart, the adrenal glands showed a higher efflux rate (Supporting data in Tables

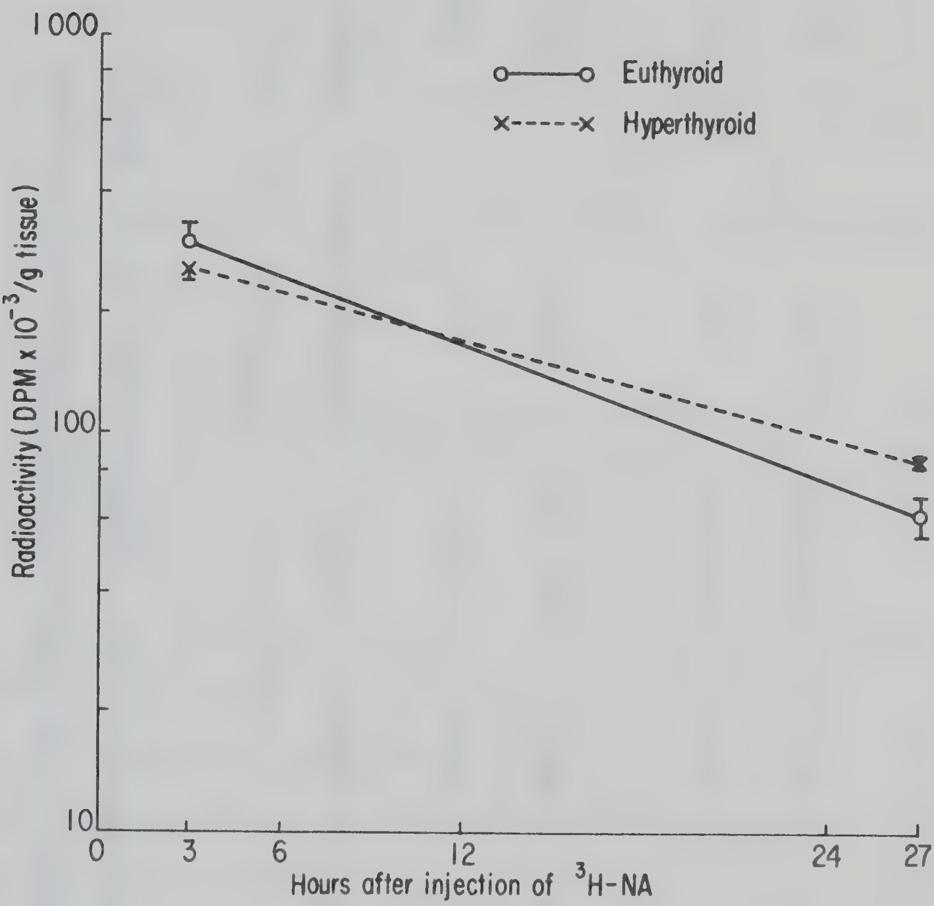


Fig. 7. Accumulation and loss of ^{3}H-NA by rat hearts under different thyroid states (Experiment A_2). Hyperthyroid and normal control rats received 100 μc/kg of ^{3}H-NA via the tail vein, and were sacrificed 3 and 27 hours after injection. Each point represents the Mean±S.E. of determinations from 6 to 7 rats. Data for this figure are tabulated in Table XVII of the Appendix.

TABLE VI

RADIOACTIVITY OF RAT TISSUES AT VARYING TIMES AFTER INJECTION

(Thyroxine administration: 100-200 μg/kg/day for 8-7 weeks)

		DPMx10^{-3}/g			
Time (Hours)	Thyroid State	Heart	Brain	Salivary Gland	Adrenal Gland
3	Euthyroid	302±40*	0.9±0.11	62±7	158±20
	Hyperthyroid	259±12	0.9±0.12	68±5	164±13
27	Euthyroid	64± 9	0.4±0.03	17±3	104± 4
	Hyperthyroid	89± 2†	0.3±0.03	18±3	82± 5†

* Mean±S.E.

† $p<0.05$.

XVII and XVIII in the appendix).

The results on the accumulation of ^{3}H-NA in the tissues of rats given 400-800 μg/kg/day of thyroxine for 2-3 weeks are given in Table VII. When the tissue contents of ^{3}H-NA were plotted against time, the fall in radioactivity was approximately linear and parallel for the normal controls and hyperthyroid animals, showing no remarkable change in the efflux rate from those reported above for the lower dose of thyroxine (Supporting data in Tables XIX and XX in the appendix).

B. Experiment I. Catecholamine Content and Accumulation and Loss of Tritiated Noradrenaline by Tissues of Hyper, Hypo and Euthyroid Rats Treated for 8 Weeks

In order to keep more uniform conditions between the three groups of rats in this experiment, thyroidectomized animals were used for both the hyper and hypothyroid groups and sham-operated animals were used for the control group. As indicated in Table II all animals were given the same diet and drinking water. In the preliminary experiments it was found that the administration of 100 μg/kg/day of thyroxine for 8 weeks to normal animals without thyroidectomy did not cause fatalities. However, during the administration of 100 μg/kg/day of the hormone for 4 weeks to thyroidectomized animals there were some deaths. Therefore the dose was reduced to 25 μg/kg/day for the following 4 weeks. As indicated in Fig. 4 the heart rate remained significantly higher in the thyroxine treated animals and the body weights were significantly above the hypothyroid group but not above the euthyroid group.

TABLE VII

RADIOACTIVITY OF RAT TISSUES AT VARYING TIMES AFTER INJECTION

(Thyroxine administration: 400-800 μg/kg/day for 3-2 weeks)

Time (Hours)	Thyroid State	DPMx10^{-3}/g Heart	Brain	Salivary Gland	Adrenal Gland
3	Euthyroid	150±12*	0.5±0.08	43±3	82±11
	Hyperthyroid	139± 7	0.7±0.04	29±3†	53± 9
27	Euthyroid	19± 3	0.7±0.08	4±0.5	38± 4
	Hyperthyroid	24± 4	0.5±0.04	2±1.1†	33± 3

* Mean±S.E.

† $p<0.05$.

At the end of the eight week period of treatment all animals were given a 100 μc/kg dose of ^{3}H-NA by tail vein. Each group of animals was divided into five sub-groups which were killed 15 minutes, 3, 6, 12 and 24 hours after the ^{3}H-NA was administered. The hearts, brains, salivary glands, adrenal glands and vasa defferentia were removed for catecholamine assay and radioactivity determinations.

The data from the catecholamine determinations are illustrated in Fig. 8 and the supporting data is in Table XXI of the Appendix. As the catecholamine content of the tissues did not show any significant differences between the various timed groups the table shows the mean ± standard error of all the tissues from each thyroid group, without consideration for the subgrouping. The A in the hearts of the hyperthyroid animals was significantly lower than in the hearts of the euthyroid animals but the tissue weights indicate some hyperplasia. In the vasa defferentia the situation was reversed and the tissue contents of A and NA in the hyperthyroid rats were significantly above the controls but the tissue weights were also lower. In the adrenal glands the A appeared slightly but not significantly higher in the hyperthyroid rats while the NA was significantly lower. There was no evidence of hyperplasia in the adrenal glands of the hyperthyroid animals.

In contrast to the hyperthyroid rats the A of the vasa defferentia and the adrenal glands of the hypothyroid animals was significantly higher than the controls as was the NA of the heart, vasa defferentia and the adrenal glands. The tissue weights of the hypothyroid animals were consistently significantly lower than the euthyroid or hyperthyroid animals as were their total body weights.

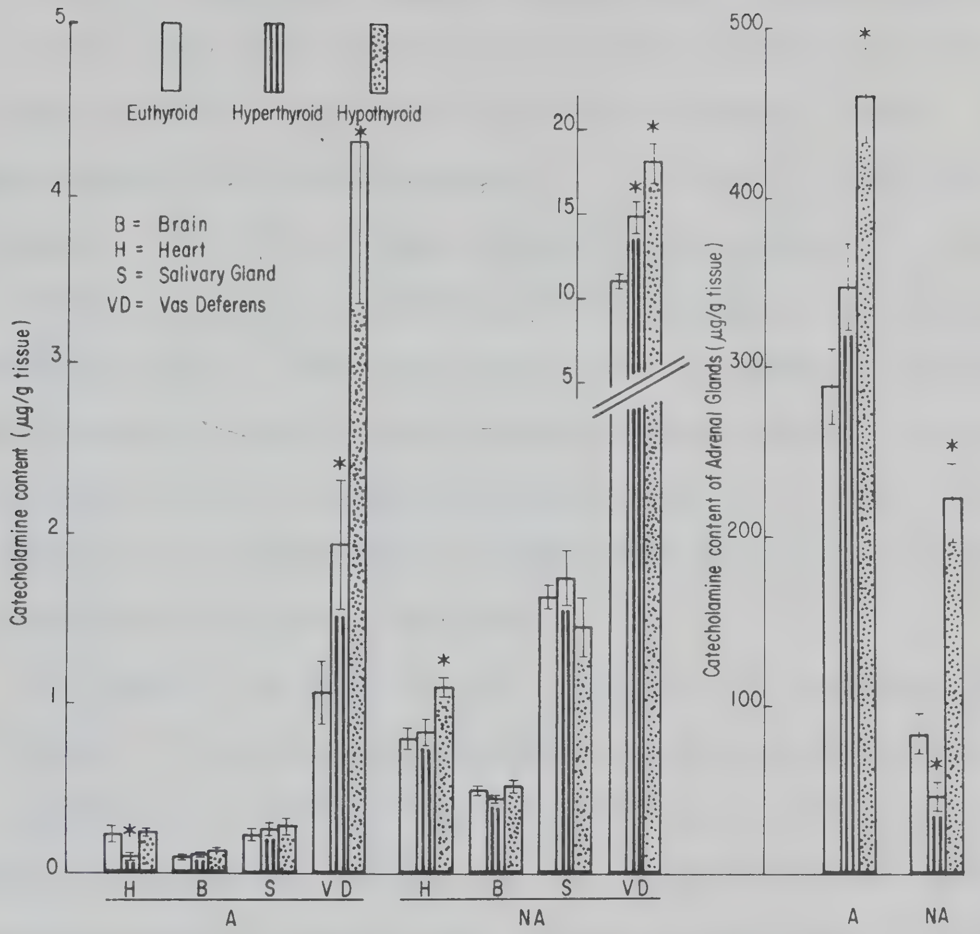


Fig. 8. The influence of the dose of thyroxine and the duration of treatment on A and NA contents in rat tissues (Experiment I). Age-matched thyroidectomized rats were treated with 100 μg/kg/day of thyroxine for 4 weeks; then the dose was reduced to 25 μg/kg/day for the following 4 weeks. The hypothyroid rats were thyroidectomized and they, as well as the sham-operated control rats received 1 ml/kg/day of alkaline saline solution.

I = S.E. of the mean; * indicates $p<0.05$, treated vs euthyroid controls. No. of animals: 22-25/group. Data for this figure are tabulated in Table XXI of the Appendix.

Table VIII shows the radioactivity per gram of the tissues measured at various times after injection of the ^{3}H-NA, and Fig. 9 and 10 are the efflux curves for the ^{3}H-NA from hearts and adrenal glands. Further supporting data from these experiments is in Tables XXII and XXIII of the Appendix. Fig. 9 and 10 show essentially the same kind of information. That is, there is no significant difference between the accumulation and efflux rates of ^{3}H-NA under hyperthyroid and euthyroid conditions but under hypothyroid conditions the uptake is reduced and the efflux rate is increased during the first 6-12 hours after injection of ^{3}H-NA, but subsequently falls off to approach or to be below the efflux rates of ^{3}H-NA from the euthyroid and hyperthyroid groups.

The data for the other tissues (Table VIII) did not show a consistent pattern. In both the salivary glands and the vasa defferentia the initial accumulation of ^{3}H-NA was significantly higher in the hyperthyroid group than in the euthyroid controls, but at subsequent times this difference was not statistically significant. In the salivary gland of the hypothyroid group the ^{3}H-NA content was not significantly different from the controls but in the vasa defferentia the hypothyroid groups were significantly higher at the 6 and 12 hour periods. As was to be expected due to the blood-brain barrier, the radioactivity of the brain was very low compared with peripheral tissues, having less than 1% of the radioactivity of the heart and less than 4% of the radioactivity of the salivary gland. As the radioactivity at various time periods was erratic it may be that it simply reflects the trapping of labelled metabolites in the tissues of the brain.

TABLE VIII

RADIOACTIVITY OF RAT TISSUES AT VARYING TIMES AFTER INJECTION

(Thyroxine administration: 25-100 µg/kg/day for 8 weeks)

Time (Hours)	Thyroid State	Heart	Brain	Salivary Gland	Adrenal Gland	Vas Deferens
		DPMx10^{-3}/g				
¼	Euthyroid	456±50*	3.3±0.07	82±15†	316±81	54±12†
	Hyperthyroid	414±30†	3.0±0.42	148±15†	318±30	100± 7†
	Hypothyroid	289±48†	3.4±0.73	61± 5	179±23	61±14
3	Euthyroid	306±17	2.8±0.28	75± 8	152±25†	36± 2
	Hyperthyroid	359±14†	6.7±0.48	88± 3	231±15†	39± 2
	Hypothyroid	210±19†	2.1±0.23	57± 4	162±19	48±10
6	Euthyroid	316±30	2.1±0.13	63± 4	182±13	16± 0.7
	Hyperthyroid	278±19†	3.0±0.06	77±19	191±22	36±13†
	Hypothyroid	77± 8†	2.0±0.08	53± 8	130±40	23± 3†
12	Euthyroid	187±14	1.1±0.11	34± 4	150± 7	26± 1
	Hyperthyroid	183± 6†	2.5±0.25	37± 2†	159±10†	31± 2†
	Hypothyroid	55± 6†	2.0±0.16	17± 4†	68±12†	38± 4†
24	Euthyroid	119± 7	2.7±0.13	15± 4	121±10†	21± 2†
	Hyperthyroid	120± 5†	2.3±0.27	12± 2	87± 8†	15± 2†
	Hypothyroid	22± 2†	1.8±0.09	14± 2	63± 7†	17± 1

* Mean±S.E.

† $p<0.05$.

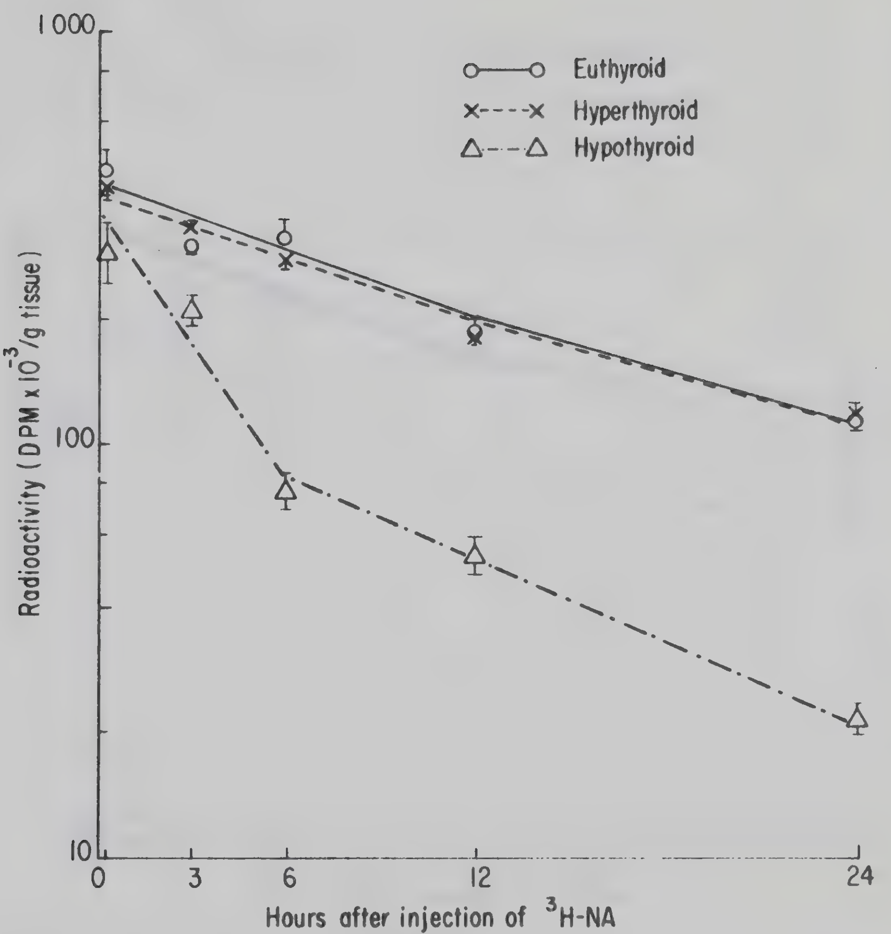


Fig. 9. Accumulation and loss of ^{3}H-NA by rat hearts under different thyroid states (Experiment I). Hyperthyroid, hypothyroid and sham-operated control rats received 100 μc/kg of ^{3}H-NA via the tail vein, and were sacrificed 15 minutes, 3, 6, 12 and 24 hours after injection. Each point represent the Mean±S.E. of determinations from 4 to 5 rats. Data for this figure are tabulated in Table XXII of the Appendix.

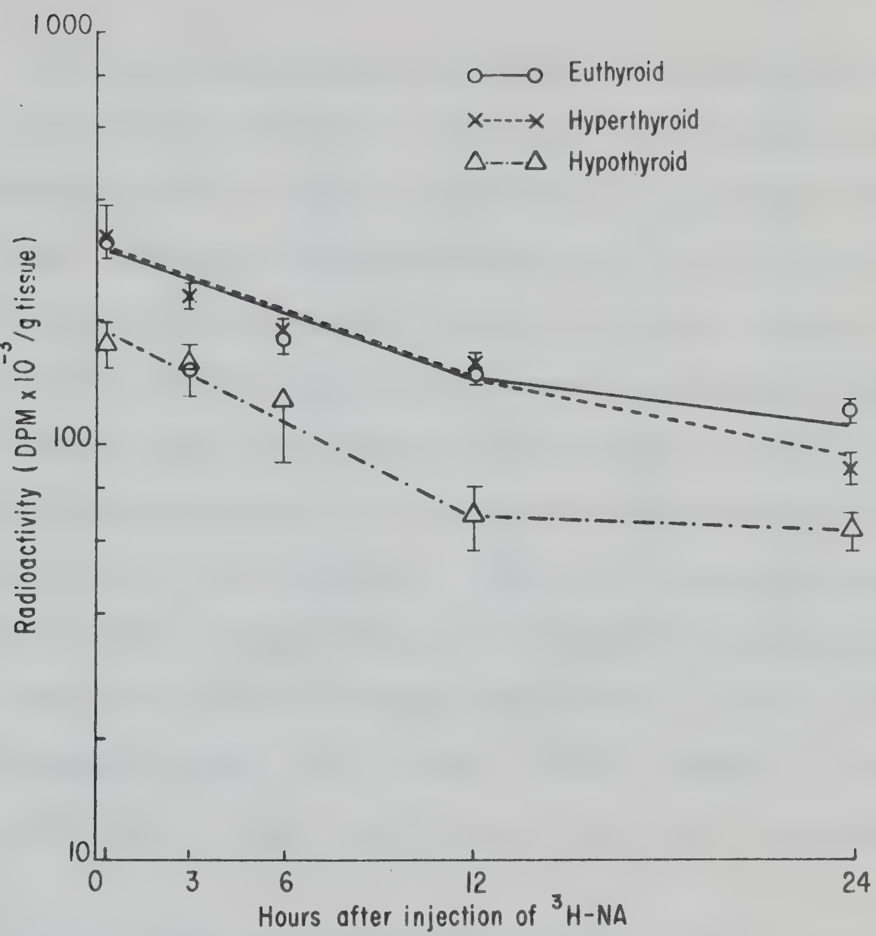


Fig. 10. Accumulation and loss of ^{3}H-NA by rat adrenal glands under different thyroid states (Experiment I). Hyperthyroid, hypothyroid and sham-operated control rats received 100 μc/kg of ^{3}H-NA via the tail vein, and were sacrificed 15 minutes, 3, 6, 12 and 24 hours after injection. Each point represents the Mean±S.E. of determinations from 4 to 5 rats. Data for this figure are tabulated in Table XXIII of the Appendix.

C. Experiment II. Catecholamine Content and Turnover of Tritiated Noradrenaline in Tissues of Hyper, Hypo and Euthyroid Rats Treated for 12 Weeks

Thirty-six sham-operated and 98 thyroidectomized, age-matched rats were used in this experiment. Fifty-four thyroidectomized animals were treated daily with 25 μg/kg of thyroxine and constituted the hyperthyroid group. Forty-four thyroidectomized animals were injected daily with the alkaline saline medium but given no thyroxine, and used as the hypothyroid group, while 36 sham-operated animals were maintained as a euthyroid control group, and similarly injected with the alkaline saline medium. All animals were fed on the low iodine diet and had 37.5 μg of iodine per 100 mls of drinking water. These rats were maintained on these regimes for 12 weeks. Sample groups were periodically weighed and tested for their heart rates, and all animals were similarly tested on the 12th week as indicated in Tables XXIV and XXV of the Appendix. Figure 11 indicates the changes in heart rate and body weight over the period of treatment.

The body weights of the hypothyroid rats did not increase significantly during the period of treatment but those of the hyperthyroid and euthyroid animals increased by approximately 100%. The heart rates of the thyroidectomized animals were all lower than the sham-operated controls at the beginning of the experiment but, while the heart rates of the hypothyroid animals continued to fall those of the hyperthyroid group rose to be significantly higher than those of the control group.

At the end of the 12 week period of treatment all the animals

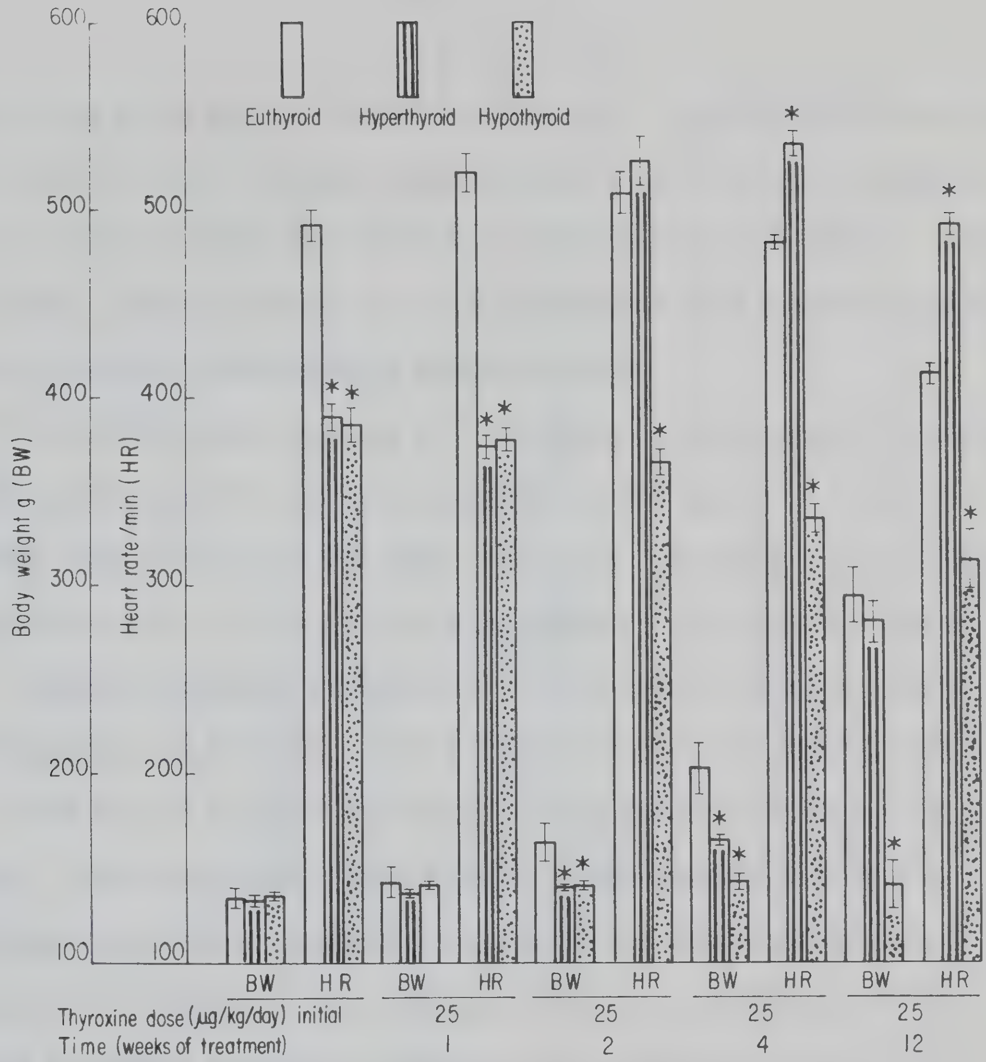


Fig. 11. The influence of the dose of thyroxine and the duration of treatment on the body weights and heart rates in rats (Experiment II). Three groups of age-matched rats were used for hyper, hypo and euthyroid controls. The hyperthyroid group were thyroidectomized and treated with 25 µg/kg/day of thyroxine for 12 weeks. The hypothyroid group were thyroidectomized and treated with 1 ml/kg/day of alkaline saline solution. The euthyroid control groups were sham-operated but were treated daily with the alkaline saline injection comparable to the other groups. I = S.E. of the mean; * indicates $p<0.05$, treated vs euthyroid controls. No. of animals: 6-12/group. Data for this figure are tabulated in Tables XXIV and XXV of the Appendix.

were given a 100 μc/kg dose of ^{3}H-NA by tail vein. Each of the three groups of animals were divided into five subgroups which were killed 15 minutes, 3, 6, 12 and 24 hours after the ^{3}H-NA was administered. The hearts, brains, salivary glands, adrenal glands and vasa defferentia were removed for catecholamine assays and radioactivity determinations.

The catecholamine content did not change significantly in each of the time periods for the various subgroups so the mean ± S.E. of the larger groups were determined for each tissue and illustrated in Fig. 12, with supporting data in Table XXVI of the Appendix. The dopamine determinations showed insignificant amounts in all tissues except the brain and adrenal glands and therefore do not appear in Fig. 12 or Table XXVI.

There was no significant change in the content of endogenous A in the heart, brain, salivary gland, adrenal gland and vas deferens of the hyperthyroid rats when compared to those of the euthyroid animals. The content of A increased in the adrenal gland and vas deferens of the hyperthyroid rats when compared to those of the euthyroid animals. The content of A increased in the adrenal gland and vas deferens of the hypothyroid rats, but not in the heart, brain and salivary gland. The content of endogenous NA was elevated in the salivary gland of hyperthyroid animals, but no significant change was observed in the heart, brain, adrenal gland and vas deferens. The content of NA increased markedly in all of the tested tissues of the hypothyroid rats. The content of endogenous DA showed no appreciable change in the brain and adrenal of the hyperthyroid animals, but it was markedly elevated in the adrenal gland of the hypothyroid rats.

Table IX indicates the progressive changes in radioactivity per gram tissue which occurred following injection of the ^{3}H-NA, and Figs. 13

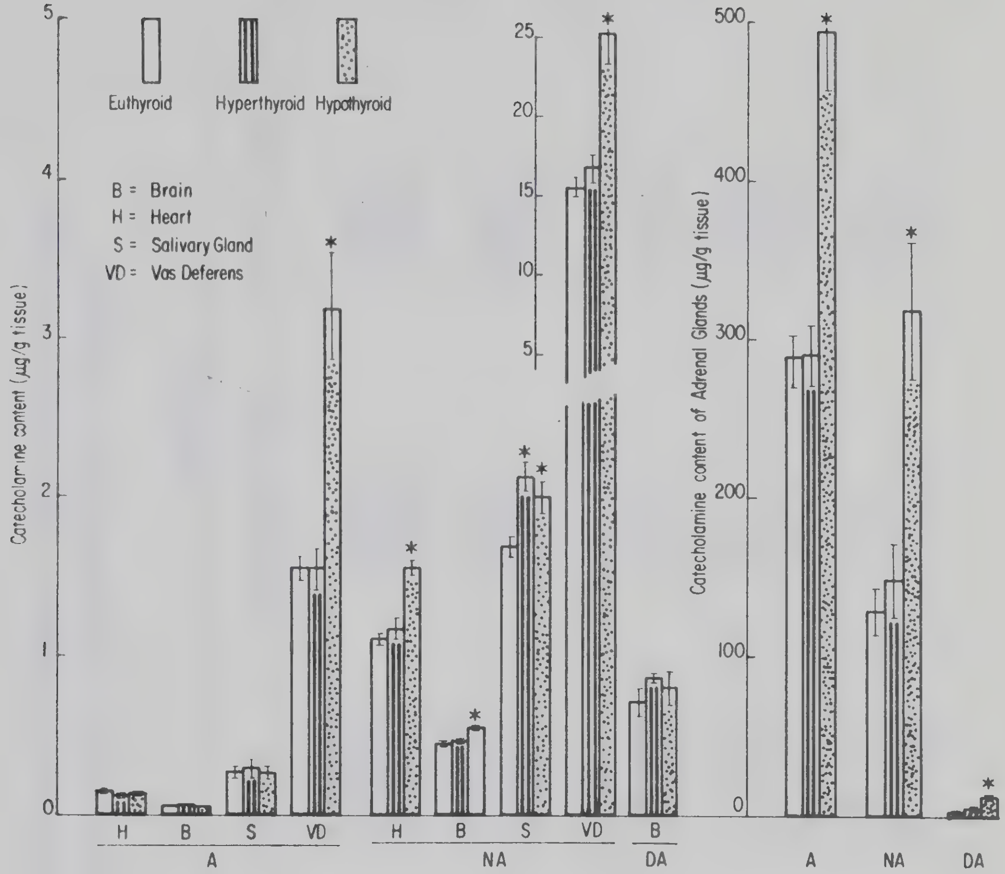


Fig. 12. The influence of the dose of thyroxine and the duration of treatment on A, NA and DA contents in rat tissues (Experiment II). Age-matched thyroidectomized rats were treated with 25 µg/kg/day for 12 weeks. The hypothyroid rats were thyroidectomized and they, as well as the sham-operated control rats received 1 ml/kg/day of alkaline saline solution. I = S.E. of the mean; * indicates $p<0.05$, treated vs euthyroid controls. No. of animals: 30/group for A and NA measurement, and 2-15/group for DA measurement. Data for this figure are tabulated in Table XXVI in the Appendix.

TABLE IX

RADIOACTIVITY OF RAT TISSUES AT VARYING TIMES AFTER INJECTION

(Thyroxine administration: 25 μg/kg/day for 12 weeks)

Time (Hours)	Thyroid State	Heart	Brain	Salivary Gland	Adrenal Gland	Vas Deferens
		$DPM \times 10^{-3}/g$				
¼	Euthyroid	1,042±59*	1.6±0.13	312±50	570±61	105±37
	Hyperthyroid	1,107±57	1.6±0.31	267±19	418±21†	71± 8
	Hypothyroid	759±57†	0.9±0.17	221±20	252±38†	71± 7
3	Euthyroid	760±33	1.3±0.32	176±35	364±13	70± 9
	Hyperthyroid	815±57	1.2±0.32	195±13	387±41	69± 6
	Hypothyroid	581±62†	1.0±0.17	190±19	217±20†	65± 6
6	Euthyroid	700±94	1.3±0.34	94± 6	365±43	59± 8
	Hyperthyroid	642±49	1.2±0.23	113±16	308±18	55± 4
	Hypothyroid	279±63†	0.8±0.11	99±14	165±25†	44± 8
12	Euthyroid	417±27	1.2±0.07	59± 5	309±23	62± 3
	Hyperthyroid	373±18	0.8±0.07	52± 1	295±19	38± 5†
	Hypothyroid	114±13†	1.0±0.27	48± 4	81±12†	36± 6†
24	Euthyroid	157±16	0.8±0.23	16± 2	194±23	26± 2
	Hyperthyroid	282±23†	0.7±0.13	16± 3	187±15	25± 1
	Hypothyroid	39±10†	0.5±0.06	22± 2	78±10†	13± 2†

* Mean±S.E.

† $p<0.05$.

and 14 show the efflux of tritium from the hearts and adrenal glands of the hyper, hypo and euthyroid animals. Further supporting data is in Tables XXVII and XXVIII of the Appendix. As found in the previous experiment (Fig. 9) the accumulation of ^{3}H-NA in the hearts of the hypothyroid animals was below those of the hyperthyroid and euthyroid animals, and the rate of efflux, as indicated by the slopes of the efflux curves, was distinctly greater during the first 12 hours, but approximately the same as the control group during the second 12 hours.

Comparing the accumulation and efflux of ^{3}H-NA by the hearts of hyperthyroid with the data from euthyroid rats (Fig. 13) it can be seen that the first four pairs of points on the curves are not significantly different but the points in the last pair are significantly different. In general these data are in agreement with that from Experiment I and, with the exception of the last point, indicates that there is no significant difference in either the acumulation or efflux rates of the ^{3}H-NA in the hearts of hyper and euthyroid rats.

Fig. 14 indicates that the accumulation and efflux patterns of ^{3}H-NA in the adrenal glands of hyper, hypo and euthyroid rats are very similar to those in the heart, the notable differences induced by changes in thyroid states being the reduced accumulation and increased efflux rates in the hypothyroid animals.

In the salivary glands and vasa defferentia no significant difference occurred in the patterns of accumulation and efflux of ^{3}H-NA in altered thyroid states. In the brain, as in Experiment I, the uptake was very low compared with the other adrenergically innervated tissues but, consistent with the others, the radioactivity in the brain tissues from

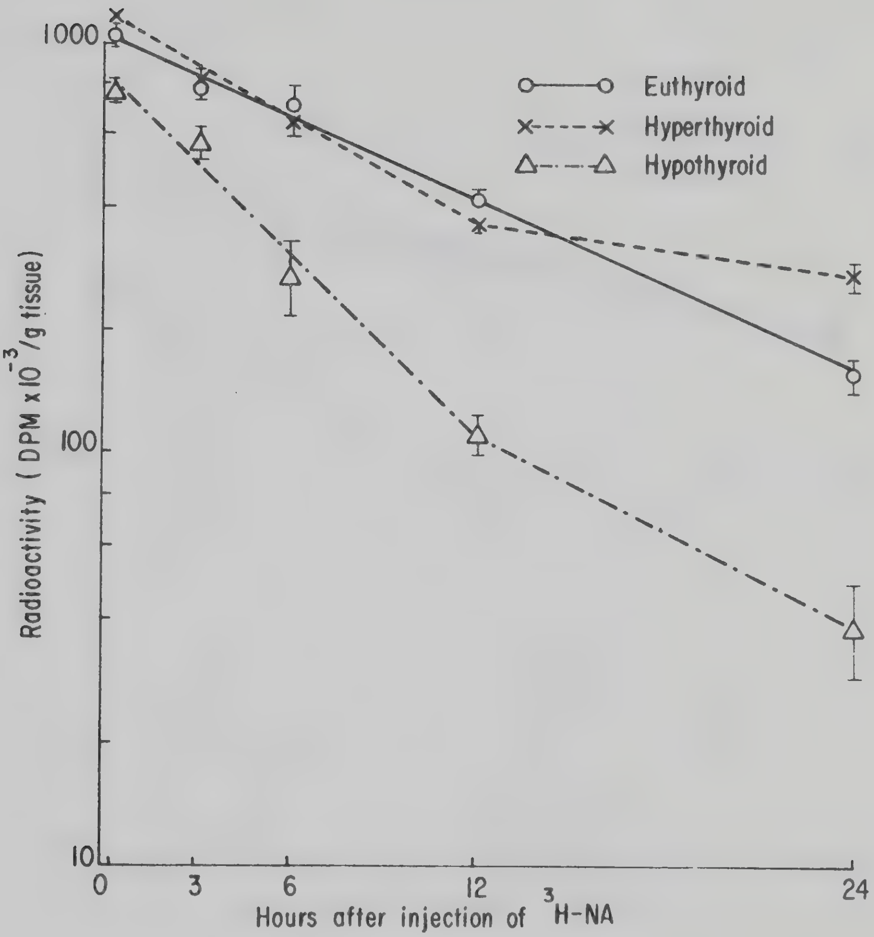


Fig. 13. Accumulation and loss of ^{3}H-NA by rat hearts under different thyroid states (Experiment II). Hyperthyroid, hypothyroid and sham-operated control rats received 100 μg/kg of ^{3}H-NA via the tail vein, and were sacrificed 15 minutes, 3, 6, 12 and 24 hours after injection. Each point represents the Mean±S.E. of determinations from 6 rats. Data for this figure are tabulated in Table XXVII of the Appendix.

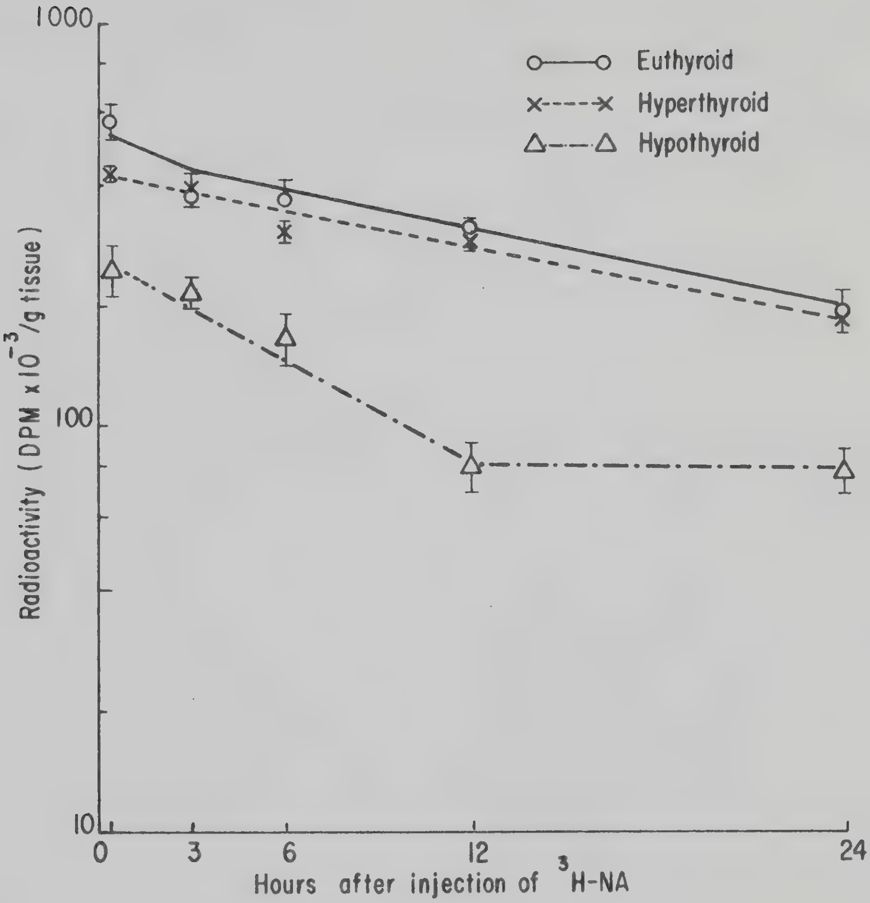


Fig. 14. Accumulation and loss of ^{3}H-NA by rat adrenal glands under different thyroid states (Experiment II). Hyperthyroid, hypothyroid and sham-operated control rats received 100 μc/kg of ^{3}H-NA via the tail vein, and were sacrificed 15 minutes, 3, 6, 12 and 24 hours after injection. Each point represents the Mean±S.E. of determinations from 6 rats. Data for this figure are tabulated in Table XXVIII of the Appendix.

hypothyroid animals was lower than that in the hyper and euthyroid animals.

DISCUSSION

The objective of this work was to investigate the possibility that the observed elevated responses to catecholamines in hyperthyroid animals and human patients may be due to altered activity in the adrenergic nervous system, in contrast to or in addition to increased sensitivity of the responding systems.

In most investigations using animal experimentation, the hyperthyroid state was induced by giving large doses of thyroxine for relatively short periods, whereas in humans the disease states are usually chronic and characterized by slow development of either hyper or hypothyroidism. For this reason it was felt to be desirable to develop the hyperthyroid state in the experimental animals by relatively low doses of thyroxine over a prolonged period of time to simulate the chronic state of the disease. In addition it has been shown by Zaimis et al. (1969) that low doses of thyroxine administered to cats over a prolonged period induced histological changes in the muscles which may be responsible for their hyperresponsiveness to adrenergic stimulation. In view of this it was thought that if part of the increased response was due to activity on the neuronal side of the neuro-effector junction this also may be more effectively demonstrated by simulating the chronic state of the disease by giving low doses for a prolonged period.

For this work it was essential to first establish criteria for hyper and hypothyroidism and the dose level of thyroxine which would develop symptoms of hyperthyroidism that could be maintained for a prolonged period without a high mortality rate. Although the classical method of

evaluating hyperthyroidism in animals has been to observe the increase in metabolic rate as indicated by increased oxygen utilization, it was felt that for this work, in which particular attention was paid to the relationship between hyperthyroidism and the responses to adrenaline and noradrenaline, it would be desirable to consider the cardiovascular responses. Because an elevated heart rate is one of the symptoms of hyperthyroidism it was decided to use the method of Barker et al. (1965) based on elevated heart rates to assess the induction of the hyperthyroid state. The preliminary experiments were therefore concerned with testing the correlation between elevated heart rate as a result of thyroxine treatment and other symptoms of hyperthyroidism, the elevated oxygen consumption and body temperature.

The heart rates of rats were usually high at the beginning of the experiments but they decreased within one or two weeks after the rat arrived in the laboratory, and then showed fairly constant values (Table XIV of the Appendix). This effect may have been caused by transportation and adaptation of the rat to its new environment. The rats appeared excited when they reached the laboratory but after approximately one week they were calm and less excitable.

Fig. 2 and 4 indicated that at the end of the period of treatment with thyroxine the body weights of the rats were a little less than those of the euthyroid animals, which was expected, due to the increased metabolic rate caused by thyroxine treatment. On the other hand the body weights of the thyroidectomized animals in the hypothyroid group were usually lower than the euthyroid controls and remained relatively constant, probably because of the removal of the thyroid gland which contributes to growth.

In the preliminary experiments there was some variation in the measurement of oxygen consumptions, rectal temperatures and heart rates. The heart rates of normal rats reported in Table III are lower than those of normal animals in the second tests (Table X in the Appendix). This was probably due to the anesthetic, as sodium barbital (250 mg/kg) was used in the former experiment and ether in the latter. Similarly the rectal temperature of the normal rats in the first measurement (Table III) appeared to be lower than those of the normal rats in the second experiment (Table X). This effect may also have been caused by the depth of anesthesia but it could have been due to the recording instrument, which was changed between the first and second experiment. In the second experiment the anesthetic used for measurement of rectal temperature was again sodium barbital but at a lower dose level (200 μg/kg). In subsequent experiments ether anesthetic was used as this was easier to apply, caused fewer deaths and the results of temperature and oxygen utilization measurements were more uniform. These preliminary experiments indicated that the heart rates and body weights were useful indicators of the change in thyroid state induced by the experimental conditions.

In Experiment A_2 the hyperthyroidism was induced by administration of thyroxine to normal rats without thyroidectomy, and they were fed on normal fortified laboratory diet. On the other hand, in Experiments I and II the hyperthyroidism was induced by administration of thyroxine to thyroidectomized rats, which were maintained on low iodine diet and iodine-containing drinking water. The purpose of this change was to attempt to keep the hyperthyroid rats under as close as possible to the same experimental conditions as the hypo and euthyroid animals. The content of the endogenous

catecholamines in the tissues of the normal rats made hyperthyroid tended to be lower than that in the euthyroid controls (Fig. 5 and 6, Experiment A_2), whereas in the thyroidectomized animals made hyperthyroid (Fig. 8 and 12, Experiments I and II) this tendency was not present and the control of catecholamines in their tissues was approximately the same as in their euthyroid controls. These data suggest that the thyroid gland in the rats plays a role in the control of synthesis of catecholamines in the adrenergically innervated tissues.

The pituitary-thyroid axis with its negative feed-back relation has led to the concept of a control system involving a hypothalamic-pituitary-thyroid triangle. The hypothalamus secretes thyrotrophin-releasing factor (TRF), which stimulates the pituitary to release TSH; this, in turn activates the thyroid follicular cells to synthesize and release thyroid hormone. A feed-back control is exerted at the pituitary level and also may be exerted at the hypothalamus by the plasma level of circulating thyroid hormones (Reichlin, 1971). It has been found that adrenaline and other hormones including thyroxine, TSH and possibly TRF all elevate levels of cyclic AMP in various tissues, possibly by activating adenyl cyclase, suggesting that cyclic AMP is the intracellular mediator of the action of many hormones. In the thyroid, TSH rapidly elevates tissue levels of cyclic AMP by activation of the enzyme, adenyl cyclase (Pastan, 1971), and the released cyclic AMP promotes synthesis of the thyroid hormones. TRF may also act in the pituitary by activating adenyl cyclase resulting in release of cyclic AMP and TSH. When thyroid hormones are released into the blood stream there is a feed-back mechanism which controls further release of TSH, possibly by an action on adenyl cyclase, controlling the release of

cyclic AMP (Robison *et al*., 1971). These authors also suggest that thyroid hormones may also exercise a feed-back control effect on the release of TRF in the hypothalamus, again, possibly through the adenyl cyclase-cyclic AMP system.

The hypothalamus contains considerable NA and is a centre for higher control over the sympathetic nervous system. The stimulation of the hypothalamus produces increased secretion of A and NA from the adrenal medulla of dogs (Goldfien and Ganong, 1962). Also stimulation of various parts of the hypothalamus produces a rise in blood pressure, pupillary dilatation, piloerection and other signs of diffuse adrenergic discharge (Ganong, 1967). Thus, the release and probably synthesis of NA in peripheral adrenergic nerves are influenced by the hypothalamus. Since thyroid hormones probably regulate some functions of the hypothalamus by their feed-back mechanism, the thyroid hormones may influence the center of the sympathetic nervous system. The removal of the thyroid gland from the rat induces a blockade of the feed-back mechanism of the thyroid hormones to the hypothalamus. As a result, the release of NA from the sympathetic nerves may be changed. Therefore, this factor may account for some differences of the experimental data between the hyperthyroid rats with the thyroid glands intact (Experiment A_2) and the hyperthyroid animals without the thyroid glands (Experiments I and II).

In the Experiment A the rats were treated in two ways. The one group was administered with a relatively small dose (100-200 μg/kg/day) of thyroxine for a longer period (7-8 weeks), and the other group was administered with a relatively large dose (400-800 μg/kg/day) of thyroxine for a shorter duration (2-3 weeks). By comparison of Table XVII and Table XIX

it can be seen that the radioactivity of the hearts of the former was much higher than that of the hearts in the latter group. That is, the former group showed a larger accumulation of ^{3}H-NA than the latter group. Similar conditions occurred in the adrenal and the salivary glands, as indicated in Tables XVIII and XX. This suggests that the administration of relatively small amount of thyroxine for a longer period induced a more marked change in rat tissues than the administration of relatively large amount of the hormone for a shorter duration. A similar example of that type of effect was reported by Schildkraut et al. (1971) who showed differences between the effects of acute and chronic administration of imipramine and protriptyline on the rate of disappearance of ^{3}H-NA from the brain as well as on the content of endogenous NA in the brain. They also showed that thyroxine influenced the rate of loss of ^{3}H-NA caused by imipramine by causing the loss of ^{3}H-NA to occur sooner. No remarkable differences were noted between the Experiment I (thyroxine 100 µg/kg/day for 4 weeks, and 25 µg/kg/day for the following 4 weeks) and Experiment II (thyroxine 25 µg/kg/day for 12 weeks), with respect to NA content in the tissues or the accumulation of ^{3}H-NA or its rates of efflux from the hearts or adrenal glands. In these experiments both groups were given relatively low doses of thyroxine for prolonged periods.

There is a possibility of thyroxine synthesis in extrathyroidal tissues of thyroidectomized rats, as thyroxine can be synthesized in the rat totally devoid of thyroid tissue after the administration of a large dose of iodide (Purves and Griesbach, 1946; Hum et al., 1951). Moreover, there is also the possibility of thyroxine-like effect of a large dose of iodide on tissues in completely thyroidectomized rats (Taurog et al., 1961,

and Evans et al., 1966). However, in the present study the amount of iodide which was given to the thyroidectomized rats is equivalent to approximately 1/20-1/100 of the amount which other investigators have used (Purves and Griesbach, 1946; Hum et al., 1951) and, no increase in the heart rate and body weight was observed in the hypothyroid groups. Therefore, in the present study the extrathyroidal synthesis of the thyroid hormone or thyroxine-like effect of iodide is not likely to have occurred.

The experimental results (Fig. 13 and 14) show that for 12 hours after the administration of ^{3}H-NA, the efflux rate in the heart and adrenal gland of the hypothyroid rats is greater than that of the sham-operated rats, although the initial accumulation was smaller in the hypothyroid animals. Montanari et al. (1963) reported that at equilibrium, the slope of the exponential decline in radioactivity caused by disappearance of ^{3}H-NA from the hearts was related to the rate of NA synthesis. Catecholamine determinations showed that during the course of the experiment there was no remarkable change in the endogenous NA contents of these tissues, but the amount of endogenous NA was markedly greater in the hypothyroid animals than that in the control rats. This fact was observed in both Experiments I and II, as were the increased efflux rates which appear to indicate an elevated NA turnover and synthesis in the hearts and the adrenals of the hypothyroid rats. This increased rate of turnover may be due to a disturbance in the storage mechanism due to the labelling dose, but if so, it is interesting that there was so little evidence of such a disturbance in the hyper and euthyroid animals. Also, the content of endogenous NA increased in the tissues of hypothyroid animals. Therefore, it is not likely that a defect in the storage mechanism had occurred.

Margolius and Gaffney (1964) showed that in dog heart atrial NA concentrations were reduced below control levels in both the hyper and hypothyroid animals. In the present study the NA content of the whole heart in the hyperthyroid rats was the same, μg/g tissue, as that of the euthyroid animals, but it was increased on a per heart basis. In further contrast to their work, the present experiments with rats showed that the NA content of the hearts in the hypothyroid animals also increased. Such differences may come from species differences, site of tissue (atrium vs atrium + ventricle) and experimental procedure. In addition, although it is not clear from their writing, the samples of atrium were probably obtained after stimulation of the cardioaccelerator nerve.

Dengler (1961) reported that heart slices from hyperthyroid rats contained less ^{3}H-NA after incubation than those from his controls, and he concluded that thyroxine, like cocaine, inhibited the uptake of catecholamines by the heart. The results obtained in the present study do not agree with the finding of Dengler. This difference may be caused by differences in experimental procedure but it may also be caused by the degree of hypertrophy in the hyperthyroid hearts as he used very large doses (4-6 mg/kg) of thyroxine. It is possible that in Dengler's experiments the sympathetic innervation was diluted in rapidly enlarging hearts.

The present study showed that turnover of ^{3}H-NA increased in the hearts and adrenal glands of hypothyroid rats. This is in agreement with the observations of Lipton _et al_. (1968), who showed that thyroid deficiency was associated with an increase in cardiac NA turnover in rats and also that there was a marked acceleration of the synthesis of ^{14}C-NA from its precursor. Although Prange _et al_. (1970) reported that acute hyperthyroidism

in rats decreased the release as well as the synthesis of NA, the present study indicates no increase in the turnover of ^{3}H-NA in hyperthyroid rats. Such a difference is puzzling, but there may be some explanations including the small number of animals tested in Prange's experiments, and the nature of the hyperthyroidism induced, which in his experiments was caused by large doses (0.5-1.25 mg/kg) of thyroxine given for only 10 days. Such very large dosage in no way simulates the chronic state of the disease in human patients, and may cause much more drastic physiological disturbances.

Data from the present experiments showed that in the heart, brain, salivary gland, adrenal gland and vas deferens, the content of endogenous NA increased in the hypothyroid rats. In addition, the ^{3}H-NA content fell more rapidly than in the hyper or euthyroid animals suggesting that the efflux of ^{3}H-NA increased from the hearts and adrenal glands of the hypothyroid rats. Therefore, it appears that the NA turnover may have increased in the sympathetic nervous system of the hypothyroid rats, but was unchanged in the hyperthyroid animals. To explain the increased NA content and turnover in hypothyroid animals three possibilities exist. These are:

1. Alteration in the receptor response
2. Response to a stressful situation
3. Increased NA synthesis due to reduction in iodotyrosine inhibitors of tyrosine hydroxylase.

The increase of NA turnover may be interpreted as evidence of an increased NA synthesis in the sympathetic nervous system, which is supported by the observations of other workers who have investigated the possibility of altered receptor responses due to changes in thyroid states.

There is evidence in the literature both supporting and opposed

to the concept of altered adrenergic receptor sensitivity induced by changes in thyroid activity. Hornbrook et al. (1965) reported that thyroid hormone produces a potentiation of catecholamine effects by causing an increased sensitivity of the myocardial catecholamine receptor site in rats. Harrison et al. (1967) showed that hyperthyroidism in man is not accompanied by an obligatory increase in the activity of the sympathetic nervous system, and they suggested that sensitization of the cardiovascular and metabolic responses to normal catecholamine levels occurs in hyperthyroidism. Prange et al. (1970) hypothesized that in hyperthyroidism receptor sensitivity is increased while the converse obtains in hypothyroidism. They showed that in the hyperthyroid rat, increased receptor sensitivity causes increased sympathetic response, which in turn tends to diminish sympathetic neuronal activity, and they postulated that diminished catecholamine synthesis would be a final expression of this tendency. This hypothesis may be supported by the finding of Dairman et al. (1968) who have shown that the rate of catecholamine synthesis varies with nerve activity and that when alpha adrenergic blocking agents are employed the synthesis of noradrenaline from ^{14}C-tyrosine was increased in the heart, brain and adrenal glands of rats. They reported that their findings with the blocking agents resemble those obtained with various procedures for increasing nerve activity in rats and indicate that receptor blockade also leads to increased synthesis and release of NA. This may be a mechanism to compensate for the diminished sensitivity of end-organs to the neurotransmitter. The alteration of sensitivity in receptors is also postulated in the central noradrenergic mechanisms under different thyroid states. Emlen et al. (1972) found that

spontaneous motor activity was less for hypothyroid rats than that in matched normal controls. Rats made hyperthyroid with thyroxine became hyperactive and showed increased sensitivity to the behaviorally activating effects of NA administered intraventricularly. These changes appeared to be induced by an increase or decrease in sensitivity of adrenergic receptors. It was hypothesized by that group that decreased postsynaptic activity is communicated to the presynaptic adrenergic neuron and that the resulting neural activity regulates the amount or activity of tyrosine hydroxylase in the midbrain. In thyroxine-treated rats, increased sensitivity to NA results in increased behavioral activity and an increased behavioral response to infusion of NA. Conversely, thyroid hormone deficiency attenuates the effects of catecholamines in hypothyroidism (Harrison, 1964 and Waldstein, 1966).

The results of van der Schoot and Moran (1965) did not support the concept that thyroxine alters the sensitivity of the receptor system of the heart and blood vessels to catecholamines. Other investigators reported similar results and suggested that the cardiovascular changes in thyroxine-treated animals may not be caused by an increased sensitivity of the cell to the catecholamines (Zaimis _et al_., 1965). Margolius and Gaffney (1965) reported that the treatment of dogs with thyroid hormone or ^{131}I to render them hyperthyroid or hypothyroid did not affect their arterial pressor or chronotropic response to either endogenously released or exogenously administered NA. Benforado and Wiggins (1965) showed that isolated myocardium strips from hypothyroid rats are not significantly less sensitive to the automaticity-producing or chronotropic effects of NA. Moreover, Bray (1966) demonstrated that thyroidectomy has little effect on

the adrenergic responses mediated by alpha receptors, and that the beta adrenergic response (myocardial contraction) is uninfluenced by thyroidectomy. Tommaselli et al. (1965) using autoradiographic techniques showed a preferential localization of l-thyroxine and triiodothyronine in the sino-ventricular bundle (bundle of His) of the hearts of rats. Their results suggest the possibility of direct effects of the thyroid hormones on impulse conduction in the heart. This hypothesis is supported by the experiments of Folkman and Edmunds (1962) and also Folkman and Long (1964). They have shown that a local area of hyperthyroid myocardium will act as a pacemaker in euthyroid dogs with complete heart block, when thyroid grafts are transplanted into the myocardium. Furthermore, a direct effect of thyroxine on myocardial contractility was reported. Buccino et al. (1967) demonstrated that the level of thyroid activity profoundly affects the intrinsic contractile state of cardiac muscle independently of NA. In addition, Zaimis et al. (1969) showed that the chronic administration of relatively small doses of thyroxine to cats and guinea pigs produces significant ultrastructural changes in the heart muscle before the appearance of tachycardia; thus, no definite evidence was obtained to support the view that thyroxine increases the sensitivity of the cardiovascular system to catecholamines per se. The suggestion was put forward by Ash et al. (1968) that in the thyroxine-treated animals a cell membrane alteration had taken place, possibly as a result of an inhibition of active ion transport.

It may be that the sensitivity of receptors are decreased in the hypothyroid state, and that decreased sensitivity of receptors may induce decreased sympathetic response. As a result, to compensate for the decreased response the sympathetic nervous activity may increase by reflex action.

The increase of endogenous NA content and increase of efflux rate of ^{3}H-NA in the tissues of the hypothyroid may be interpreted as the increase of NA synthesis. And the increase of NA synthesis is possibly the result of increased sympathetic nervous activity. If this happens in the hypothyroid state, it appears logical that the reverse situation would occur in the hyperthyroid state. Then the NA content in the tissues of the hyperthyroid rats would decrease while that of the hypothyroid animals increases. In the present study that relationship was not shown (Fig. 8 and 12), so that the results obtained cannot be simply explained by the change of the sensitivity of receptors. There appeared to be some other factor. A possible explanation is that the changes obtained in this experiment may be caused by some form of stress. The loss of the thyroid, an important organ for the normal existence of the animals, can be considered as a form of stress to thyroidectomized rats. This concept is consistent with the facts that various stressors such as cold environment and operations may cause a strong negative iodine balance and thus lead to a hypothyroid state (Waldenström, 1945).

It has been reported that there was a close relationship between stress and thyroid function and that stress could cause a release of catecholamines from the adrenal glands (Cannon, 1915; Williams et al., 1949; and Selye, 1950). Moreover, in a cold stress the activity of tissues was regulated by the sympathetic nervous system through the increased release of NA (Smith and Roberts, 1964; Cottle et al., 1967; Bhagat and Friedman, 1969; Thoenen, 1970; Wilson et al., 1970 and Sellers et al., 1971). After immobilization stress the level of enzymes present in the adrenal gland, tyrosine hydroxylase, dopamine-β-hydroxylase and phenylethanolamine-N-methyl

transferase which were involved in the synthesis of DA, NA and A, increased significantly in mice and rats (Kvetňanský et al., 1970a and b, 1971a, b and c, and Ciaranello et al., 1972). In the present study the content of endogenous DA, NA and A in the adrenal glands of hypothyroid rats markedly increased and the turnover of ^{3}H-NA in the hearts and adrenal glands also increased. In addition, the NA content of other sympathetically innervated tissues increased after thyroidectomy. The observed increased catecholamine content must have been caused either by increased accumulation or decreased loss but, as the accumulation of the ^{3}H-NA was lower than in the euthyroid animals, and the rate of loss was initially much higher the increased amount of endogenous NA cannot be due to slower degradation causing accumulation. However, both the higher NA content and the increased rate of loss of the label may be due to an increased rate of synthesis and turnover of the stored NA. As indicated above that type of change may be induced by stressful circumstances and in this regard the results obtained in these experiments are consistent with those of other investigators.

The hypothyroid state induced by thyroidectomy of the rats may have induced a stressful existence to which these animals responded by increased sympathetic activity resulting in both increased synthesis and turnover of catecholamines.

It has been noted that mono and diiodotyrosine are potent inhibitors of tyrosine hydroxylase, the rate limiting step in NA synthesis (Udenfriend et al., 1966). Since the inhibitory influence of these precursors of thyroid hormones were much less in the hypothyroid rats because of the removal of thyroid glands from the rats, it is possible that both the observed increased rate of loss of ^{3}H-NA (Fig. 9, 10, 13 and 14) and

the increased content of catecholamines in the tissues of the hypothyroid animals (Fig. 8 and 12) may be due to increased synthesis of NA caused by increased tyrosine hydroxylase activity. From the results obtained in the present study no remarkable changes of endogenous CA content and of efflux rate of ^{3}H-NA administered intravenously in the hyperthyroid rats was found. Therefore, these experiments do not support the suggestion that the elevated responses to adrenergic stimuli observed in hyperthyroid animals and human patients are due to altered activity in the sympathetic nervous system. These experiments do not show any alterations in the uptake, synthesis or turnover of NA at adrenergic nerve endings of the hyperthyroid animals. In the present study the increased content of catecholamines and increased turnover of ^{3}H-NA in the tissues of hypothyroid rats may be caused by a stress and/or increased acitivity of tyrosine hydroxylase in the tissues. Thyroxine appears to exert its effect by direct action to tissues and to have no influence on the function of presynaptic side of peripheral adrenergic nerves.

SUMMARY AND CONCLUSIONS

1. The influences of chronic hyper and hypothyroidism on the catecholamine contents and the accumulation and efflux of tritiated noradrenaline (^{3}H-NA) were investigated in the heart, brain, salivary gland, adrenal gland and vas deferens in rats.

2. A small dose of thyroxine increased the heart rate, oxygen consumption and body temperature in rats showing that the heart rate can be used as an indicator of an elevated thyroid state in the rats.

3. The heart rates of the hyperthyroid rats were significantly increased as compared with the euthyroid animals and they were maintained at an almost constant level as long as thyroxine was administered daily. On the other hand, the heart rates of the hypothyroid rats were significantly decreased.

4. The body weights of the hyperthyroid rats increased gradually, but were less than those of the euthyroid animals, while the body weights of the hypothyroid rats became significantly lower than the hyperthyroid animals and either did not change or decreased slightly.

5. The endogenous catecholamine content of the heart, brain, salivary gland and adrenal gland of the hypothyroid animals increased significantly but either no remarkable changes or the tendency toward a decrease in the endogenous catecholamine content was found in the same tissues of the hyperthyroid animals. The endogenous A and NA increased in the vas deferens of both the hyperthyroid and the hypothyroid rats.

6. When the tissue contents of the hearts and adrenal glands were plotted

against time, the fall in radioactivity was approximately linear and parallel for the euthyroid and hyperthyroid animals, but in the hypothyroid rats there was a break in the degradation curve, showing a more rapid fall in the first 6-12 hours, and a slow fall, approximately parallel to that of the other states, for the latter part of the test period.

7. The changes in endogenous catecholamine content and in the degradation curves of ^{3}H-NA in rat tissues suggest that in hypothyroidism there was an increased synthesis and turnover of NA in the sympathetic nerves. The mechanism involved in the regulation of NA turnover is not clearly understood, but it appears possible that the observed increased NA turnover may have been due to a compensatory mechanism to a state of stress caused by the thyroidectomy, or to an increased tyrosine hydroxylase activity due to a reduction of circulating iodotyrosine derivatives.

8. Although many of the manifestations of hyperthyroidism resemble those of sympathetic hyperactivity, no remarkable changes in uptake, accumulation and efflux rate of ^{3}H-NA were found between the hyperthyroid and the euthyroid rats. Therefore, the effect of thyroxine appears to show no relation to the functional changes of the peripheral adrenergic nerve.

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APPENDIX

TABLE X

HEART RATE, OXYGEN CONSUMPTION AND RECTAL TEMPERATURE IN RATS UNDER DIFFERENT THYROID STATES

(Thyroxine administration: 100-200 μg/kg/day for 2 weeks)

Thyroid State	Thyroxine Dose (μg/kg/day)	No. of Animals	Body Wt (g)	Heart Rate (beats/min)	O_2 Consumption (ml/min/g)	Rectal Temperature (°C)
Euthyroid		8	356±8.2*	413±12	0.017±0.0004	38.1±0.2
Hyperthyroid	100	8	315±9.5†	508±14†	0.023±0.0006†	39.0±0.2†
Euthyroid		5	346±8.8	408± 8	0.016±0.0007	37.8±0.4
Hyperthyroid	200	6	320±7.5	549±12†	0.024±0.0010†	39.3±0.1†

* Mean±S.E.

† $p<0.05$.

Temperature of Chamber = 25°C.

TABLE XI

THE INFLUENCE OF THYROIDECTOMY AND OF THYROXINE DOSE AND DURATION

OF TREATMENT ON THE BODY WEIGHTS OF RATS

(Thyroxine administration: 3-50 μg/kg/day for 23 days)

Thyroid State	Thyroxine Dose / Days of Treatment	Before Treatment 0	3 μg/kg/day 1	2	3	4	5	6	7	25 μg/kg/day 8	9	10	11
Euthyroid		209± 4.0* (14)	207± 5.7 (14)	205± 6.4 (14)	210± 6.0 (14)	219± 4.4 (13)	225± 6.6 (13)	228± 7.4 (13)	233± 8.2 (12)	233± 4.7 (11)	236± 4.7 (11)	241± 4.8 (11)	245± 5.0 (11)
Thyroxine-treated		213± 4.4 (15)	213± 3.7 (15)	217± 4.5 (15)	220± 4.5 (15)	223± 4.6 (15)	232± 5.3 (15)	232± 5.6 (15)	238± 7.4 (14)	232± 7.0 (14)	235± 8.0 (14)	235± 7.7 (14)	243± 4.6 (13)
Hypothyroid		157± 2.0† (28)	159± 1.8† (28)	158± 2.0† (28)	159± 1.9† (28)	161± 1.9† (28)	159± 2.0† (28)	158± 2.0† (28)	157± 2.0† (28)	157± 2.1† (28)	157± 2.1† (28)	159± 2.0† (28)	160± 2.1† (28)

Thyroid State	25 μg/kg/day 12	13	14	15	16	17	18	50 μg/kg/day 19	20	21	22	23
Euthyroid	251± 4.4 (11)	250± 4.8 (11)	256± 5.0 (11)	252± 2.9 (7)	250± 4.2 (7)	250± 3.3 (7)	252± 5.3 (7)	256± 6.1 (7)	266 (1)	266 (1)	273 (1)	277 (1)
Thyroxine-treated	249± 8.0 (13)	249± 9.1 (13)	252± 10.7 (13)	257± 9.0 (11)	252± 9.0 (11)	246± 10.3 (11)	248± 12.1 (11)	264± 9.9 (9)	262± 11.0 (9)	269± 9.8 (9)	270± 9.4 (9)	272± 8.3 (9)
Hypothyroid	153± 2.4† (28)	152± 2.5† (28)	153± 2.4† (27)	151± 2.4† (25)	152± 2.5† (25)	153± 2.6† (25)	152± 2.5† (25)	153± 2.5† (25)	149± 2.3 (25)	149± 2.8 (19)	150± 2.9 (19)	146± 3.9 (13)

* Mean±S.E. (weight in g).

† p<0.05.

( ) indicates No. of animals used.

TABLE XII

THE INFLUENCE OF THYROIDECTOMY AND OF THYROXINE DOSE AND DURATION OF TREATMENT ON THE HEART RATES OF RATS

(Thyroxine administration: 3-50 μg/kg/day for 23 days)

	Thyroxine Dose	Before Treatment	3 μg/kg/day	25 μg/kg/day	50 μg/kg/day
Thyroid State	Days of Treatment	0	7	11	5
Euthyroid		353±17 (14)*	381±10 (12)	347±23 (7)	438 (1)
Thyroxine-treated		365±10 (15)	386± 8 (14)	351±16 (11)	423±16 (9)
Hypothyroid		314±12 (28)†	183± 6 (28)†	202± 8 (25)†	312± 5 (13)

* Mean±S.E. (beats/min).

[First, 2nd and 3rd measurements Sodium barbital anesthesia (250mg/kg/i.p.); 4th measurement ether anesthesia].

† $p<0.05$.

( ) indicates No. of animals used.

TABLE XIII

THE INFLUENCE OF THYROIDECTOMY AND OF THYROXINE DOSE AND DURATION OF TREATMENT ON THE BODY WEIGHTS OF RATS

(Thyroxine administration: 25-100 µg/kg/day for 8 weeks)

	Thyroxine Dose	Before Treatment	100 µg/kg/day		25 µg/kg/day	
Thyroid State	Weeks of Treatment	0	2	4	6	8
Euthyroid		109±3.8 (7)*	128±5.8 (7)	150±9.1 (7)	204±3.7 (7)	240±4.9 (5)
Hyperthyroid		121±1.8 (9)	139±3.2 (7)	164±2.7 (7)	196±2.4 (7)	221±6.5 (5)†
Hypothyroid		120±1.5 (7)	128±3.2 (6)	135±3.8 (5)	141±4.5 (5)†	137±7.1 (5)†

* Mean±S.E. (Weight in g).

† $p<0.05$.

( ) indicates No. of animals used.

TABLE XIV

THE INFLUENCE OF THYROIDECTOMY AND OF THYROXINE DOSE AND DURATION OF TREATMENT ON THE HEART RATES OF RATS

(Thyroxine administration: 25-100 µg/kg/day for 8 weeks)

Thyroid State	Thyroxine Dose	Before Treatment	100 µg/kg/day		25 µg/kg/day	
	Weeks of Treatment	0	2	4	6	8
Euthyroid		473±23 (7)*	495±20 (7)	468±18 (7)	453±19 (7)	461±12 (5)
Hyperthyroid		383± 6 (9)†	533±11 (7)†	531±16 (7)†	531±10 (7)†	533±18 (5)†
Hypothyroid		350±11 (7)†	352±17 (6)†	329± 4 (5)†	341±14 (5)†	326± 8 (5)†

* Mean±S.E. (beats/min).

† $p<0.05$.

( ) indicates No. of animals used.

TABLE XV

CATECHOLAMINE CONTENT OF RAT TISSUES IN DIFFERENT THYROID STATES

(Thyroxine administration: 400-800 μg/kg/day for 3-2 weeks)

Tissue	Thyroid State	No.	Body Wt (g)	Tissue Wt (g)	A μg/tissue	A μg/g	NA μg/tissue	NA μg/g	DA μg/tissue	DA μg/g
Heart	Euthyroid	15	294±8.3*	1.01±0.02	0.09	0.09±0.01	1.01	1.01±0.10		
	Hyperthyroid	15	264±6.2†	1.21±0.03 †	0.08	0.07±0.01	1.06	0.88±0.05		
Brain	Euthyroid	15	294±8.3	1.83±0.02	0.15	0.08±0.01	1.02	0.56±0.06	2.01	1.10±0.18 (10)
	Hyperthyroid	15	264±6.2†	1.71±0.05 †	0.10	0.06±0.004	0.89	0.52±0.02	1.16	0.68±0.08 (12)†
Salivary Gland	Euthyroid	15	294±8.3	0.51±0.02	0.08	0.16±0.03	1.33	2.60±0.12		
	Hyperthyroid	15	264±6.2†	0.53±0.01	0.16	0.30±0.03†	1.29	2.44±0.13		
Adrenal Gland	Euthyroid	15	294±8.3	0.045±0.001	21.90	487±23	8.32	185±25	0.43	9.77 (1)
	Hyperthyroid	15	264±6.2†	0.057±0.002†	21.51	377±16†	5.70	100±11†	0.18	3.27±1.24 (6)†

* Mean±S.E.

† $p<0.05$.

( ) indicates No. of animals used in DA measurement.

TABLE XVI

CATECHOLAMINE CONTENT OF RAT TISSUES IN DIFFERENT THYROID STATES

(Thyroxine administration: 100-200 μg/kg/day for 8-7 weeks)

Tissue	Thyroid State	No.	Body Wt (g)	Tissue Wt (g)	A		NA		DA	
					μg/tissue	μg/g	μg/tissue	μg/g	μg/tissue	μg/g
Heart	Euthyroid	13	422±7.0*	1.25±0.03	0.16	0.13±0.02	1.24	0.99±0.09		
	Hyperthyroid	14	377±5.1†	1.55±0.03†	0.11	0.07±0.02†	1.32	0.85±0.05		
Brain	Euthyroid	13	422±7.0	1.87±0.02	0.21	0.11±0.02	1.40	0.75±0.06	1.51	0.81±0.10 (12)
	Hyperthyroid	14	377±5.1†	1.83±0.03	0.15	0.08±0.01	1.04	0.57±0.05†	1.37	0.75±0.11 (10)
Salivary Gland	Euthyroid	13	422±7.0	0.63±0.02	0.28	0.44±0.04	1.51	2.40±0.15		
	Hyperthyroid	14	377±5.1†	0.76±0.02†	0.19	0.25±0.10	1.89	2.49±0.18		
Adrenal Gland	Euthyroid	13	422±7.0	0.048±0.002	22.08	460±52	13.58	283±31	0.44	9.22±2.34 (4)
	Hyperthyroid	14	377±5.1†	0.054±0.002†	21.06	390±39	6.48	120±19†	0.19	3.49±1.12 (7)†

* Mean±S.E.

† $p<0.05$.

( ) indicates No. of animals used in DA measurement.

TABLE XVII

UPTAKE AND RETENTION OF ^{3}H-NORADRENALINE IN RAT TISSUES UNDER DIFFERENT THYROID STATES

(Thyroxine administration: 100-200 μg/kg/day for 8-7 weeks)

Hour After Injection of ^{3}H-NA	Thyroid State	No.	Body Wt (g)	Heart			Brain		
				Wt (g)	Radioactivity		Wt (g)	Radioactivity	
					DPMx10^{-3}/tissue	DPMx10^{-3}/g		DPMx10^{-3}/tissue	DPMx10^{-3}/g
3	Euthyroid	6	413± 5.1*	1.21±0.04	365	302±40	1.88±0.02	1.6	0.9 ±0.11
	Hyperthyroid	7	384± 7.2†	1.61±0.03†	416	259±12	1.79±0.05	1.6	0.9 ±0.12
27	Euthyroid	7	430±11.9	1.29±0.05	83	64± 9	1.87±0.04	0.7	0.4 ±0.03
	Hyperthyroid	7	371± 6.9†	1.50±0.04†	134	89± 2†	1.87±0.03	0.6	0.3 ±0.03

* Mean±S.E.

† $p<0.05$.

TABLE XVIII

UPTAKE AND RETENTION OF ^{3}H-NORADRENALINE IN RAT TISSUES UNDER

DIFFERENT THYROID STATES

(Thyroxine administration: 100-200 μg/kg/day for 8-7 weeks)

				Salivary Gland			Adrenal Gland		
					Radioactivity			Radioactivity	
Hour After Injection of ^{3}H-NA	Thyroid State	No.	Body Wt (g)	Wt (g)	DPMx10^{-3}/tissue	DPMx10^{-3}/g	Wt (g)	DPMx10^{-3}/tissue	DPMx10^{-3}/g
3	Euthyroid	6	413± 5.1*	0.65±0.02	40	62±7	0.047±0.003	7.4	158±20
	Hyperthyroid	7	384± 7.2†	0.80±0.02†	54	68±5	0.054±0.002	8.9	164±13
27	Euthyroid	7	430±11.9	0.62±0.03	11	17±3	0.049±0.002	5.1	104± 4
	Hyperthyroid	7	371± 6.9†	0.73±0.03†	13	18±3	0.054±0.003	4.4	82± 5†

* Mean±S.E.

† $p<0.05$.

TABLE XIX

UPTAKE AND RETENTION OF ^{3}H-NORADRENALINE IN RAT TISSUES
UNDER DIFFERENT THYROID STATES
(Thyroxine administration: 400-800 μg/kg/day for 3-2weeks)

				Heart			Brain		
					Radioactivity			Radioactivity	
Hours After Injection of ^{3}H-NA	Thyroid State	No.	Body Wt (g)	Wt (g)	DPMx10^{-3}/ tissue	DPMx10^{-3}/ gram	Wt (g)	DPMx10^{-3}/ tissue	DPMx10^{-3}/ gram
3	Euthyroid	8	305±12.7*	1.03±0.03	155	150±12	1.84±0.03	0.99	0.5 ±0.08
	Hyperthyroid	7	263± 7.8	1.22±0.05†	171	139± 7	1.72±0.07	1.14	0.7 ±0.04
27	Euthyroid	7	281± 9.1	0.98±0.03	19	19± 3	1.82±0.04	1.37	0.7 ±0.08
	Hyperthyroid	7	265±11.3	1.19±0.03†	29	24± 4	1.70±0.07	0.88	0.5 ±0.04

* Mean±S.E.

† $p<0.05$.

TABLE XX

UPTAKE AND RETENTION OF ^{3}H-NORADRENALINE IN RAT TISSUES UNDER

DIFFERENT THYROID STATES

(Thyroxine administration: 400-800 μg/kg/day for 3-2 weeks)

Hour After Injection of ^{3}H-NA	Thyroid State	No.	Body Wt (g)	Salivary Gland Wt (g)	Salivary Gland Radioactivity DPMx10^{-3}/tissue	Salivary Gland Radioactivity DPMx10^{-3}/g	Adrenal Gland Wt (g)	Adrenal Gland Radioactivity DPMx10^{-3}/tissue	Adrenal Gland Radioactivity DPMx10^{-3}/g
3	Euthyroid	8	305±12.7*	0.52±0.03	22	43±3	0.045±0.002	3.7	82±11
	Hyperthyroid	7	263± 7.8	0.53±0.02	15	29±3†	0.056±0.002†	3.0	53± 9
27	Euthyroid	7	281± 9.1	0.50±0.03	1.9	38±0.5	0.045±0.001	1.7	38± 4
	Hyperthyroid	7	265±11.3	0.54±0.02	1.2	23±1†	0.059±0.003†	1.9	33± 3

* Mean±S.E.

† $p<0.05$.

TABLE XXI

CATECHOLAMINE CONTENT OF RAT TISSUES IN DIFFERENT THYROID STATES

(Thyroxine administration: 25-100 μg/kg/day for 8 weeks)

Tissue	Thyroid State	No.	Body Wt (g)	Tissue Wt (g)	A		NA	
					μg/tissue	μg/g	μg/tissue	μg/g
Heart	Euthyroid	25	$267 \pm 6.2^{*}$	0.84 ± 0.02	0.19	0.23 ± 0.05	0.67	0.80 ± 0.06
	Hyperthyroid	22	$234 \pm 3.8^{\dagger}$	$1.19 \pm 0.02^{\dagger}$	0.12	$0.10 \pm 0.02^{\dagger}$	1.00	0.84 ± 0.08
	Hypothyroid	23	$134 \pm 3.8^{\dagger}$	$0.36 \pm 0.01^{\dagger}$	0.09	0.24 ± 0.04	0.40	$1.10 \pm 0.07^{\dagger}$
Brain	Euthyroid	25	267 ± 6.2	1.81 ± 0.02	0.18	0.10 ± 0.01	0.91	0.50 ± 0.03
	Hyperthyroid	22	$234 \pm 3.8^{\dagger}$	1.78 ± 0.03	0.20	0.11 ± 0.01	0.80	0.45 ± 0.03
	Hypothyroid	23	$134 \pm 3.8^{\dagger}$	$1.60 \pm 0.02^{\dagger}$	0.21	0.13 ± 0.03	0.83	0.52 ± 0.04
Salivary Gland	Euthyroid	25	267 ± 6.2	0.46 ± 0.01	0.11	0.23 ± 0.04	0.75	1.64 ± 0.06
	Hyperthyroid	22	$234 \pm 3.8^{\dagger}$	$0.51 \pm 0.02^{\dagger}$	0.13	0.26 ± 0.04	0.89	1.75 ± 0.16
	Hypothyroid	23	$134 \pm 3.8^{\dagger}$	$0.23 \pm 0.01^{\dagger}$	0.06	0.28 ± 0.05	0.34	1.46 ± 0.17
Adrenal Gland	Euthyroid	25	267 ± 6.2	0.043 ± 0.002	12.47	290 ± 22	3.61	84 ± 12
	Hyperthyroid	22	$234 \pm 3.8^{\dagger}$	0.041 ± 0.001	14.27	348 ± 25	1.93	$47 \pm 9^{\dagger}$
	Hypothyroid	23	$134 \pm 3.8^{\dagger}$	$0.028 \pm 0.001^{\dagger}$	12.63	$451 \pm 28^{\dagger}$	6.24	$223 \pm 21^{\dagger}$
Vas Deferens	Euthyroid	25	267 ± 6.2	0.17 ± 0.01	0.18	1.07 ± 0.19	1.87	11 ± 0.4
	Hyperthyroid	22	$234 \pm 3.8^{\dagger}$	$0.13 \pm 0.01^{\dagger}$	0.25	$1.94 \pm 0.38^{\dagger}$	1.95	$15 \pm 0.9^{\dagger}$
	Hypothyroid	23	$134 \pm 3.8^{\dagger}$	$0.07 \pm 0.01^{\dagger}$	0.30	$4.31 \pm 0.95^{\dagger}$	1.26	$18 \pm 1.2^{\dagger}$

* Mean±S.E.

† $p<0.05$.

TABLE XXII

UPTAKE AND RETENTION OF ^{3}H-NORADRENALINE IN RAT TISSUES UNDER DIFFERENT THYROID STATES

(Thyroxine administration: 25-100 μg/kg/day for 8 weeks)

Hour After Injection of ^{3}H-NA	Thyroid State	No.	Body Wt (g)	Heart Wt (g)	Heart Radioactivity DPMx10^{-3}/tissue	Heart Radioactivity DPMx10^{-3}/g	Brain Wt (g)	Brain Radioactivity DPMx10^{-3}/tissue	Brain Radioactivity DPMx10^{-3}/g	Salivary Gland Wt (g)	Salivary Gland Radioactivity DPMx10^{-3}/tissue	Salivary Gland Radioactivity DPMx10^{-3}/g
½	Euthyroid	5	240± 4.9*	0.75±0.02	342	466±50	1.73±0.05	5.7	3.3±0.07	0.51±0.02	42	82±15
	Hyperthyroid	4	223± 7.3	1.22±0.04†	505	414±30	1.66±0.15	5.0	3.0±0.42	0.53±0.02	78	148±15†
	Hypothyroid	4	137± 9.3†	0.38±0.02†	110	289±48†	1.65±0.05	5.6	3.4±0.73	0.26±0.04†	16	61± 5
3	Euthyroid	5	246±17.6	0.82±0.04	251	306±17	1.74±0.06	4.9	2.8±0.28	0.42±0.02	31	75± 8
	Hyperthyroid	4	231± 6.9	1.16±0.04†	416	359±14	1.71±0.03	11.7	6.7±0.48	0.52±0.01†	46	88± 3
	Hypothyroid	5	149±12.4†	0.40±0.04†	84	210±19†	1.56±0.08	3.3	2.1±0.23	0.22±0.02†	14	57± 4
6	Euthyroid	5	287±13.0	0.91±0.06	288	316±30	1.83±0.04	3.8	2.1±0.13	0.47±0.01	30	63± 4
	Hyperthyroid	5	227± 6.1†	1.13±0.03†	314	278±19	1.80±0.03	5.4	3.0±0.06	0.44±0.03	34	77±19
	Hypothyroid	4	132± 3.5†	0.38±0.02†	29	77± 8†	1.60±0.05†	3.2	2.0±0.08	0.23±0.01†	12	53± 8
12	Euthyroid	5	278± 3.5	0.88±0.03	165	187±14	1.86±0.02	2.0	1.1±0.11	0.49±0.01	17	34± 4
	Hyperthyroid	4	234± 6.0†	1.18±0.04†	216	183± 6	1.67±0.10	4.2	2.5±0.25	0.49±0.03	18	37± 2
	Hypothyroid	5	123± 2.5†	0.34±0.004†	19	55± 6†	1.58±0.02†	3.2	2.0±0.16	0.20±0.03†	3.4	17± 4†
24	Euthyroid	5	284±11.6	0.84±0.02	100	119± 7	1.87±0.07	5.0	2.7±0.13	0.44±0.02	6.6	15± 4
	Hyperthyroid	5	251± 9.6	1.28±0.04†	154	120± 5	1.87±0.06	4.3	2.3±0.27	0.56±0.03†	6.7	12± 2
	Hypothyroid	5	128± 6.5†	0.32±0.01†	7	22± 2†	1.62±0.04	2.9	1.8±0.09	0.21±0.01†	2.9	14± 2

* Mean±S.E.

† $p<0.05$.

TABLE XXIII

UPTAKE AND RETENTION OF ^{3}H-NORADRENALINE IN RAT TISSUES UNDER DIFFERENT THYROID STATES

(Thyroxine administration: 25-100 μg/kg/day for 8 weeks)

Hour After Injection of ^{3}H-NA	Thyroid State	No.	Body Wt (g)	Adrenal Gland Wt (g)	Adrenal Gland Radioactivity DPMx10^{-3}/tissue	Adrenal Gland Radioactivity DPMx10^{-3}/g	Vas Deferens Wt (g)	Vas Deferens Radioactivity DPMx10^{-3}/tissue	Vas Deferens Radioactivity DPMx10^{-3}/g
½	Euthyroid	5	240± 4.9*	0.038±0.003	12	316±81	0.14±0.01	7.6	54±12
	Hyperthyroid	4	223± 7.3	0.036±0.005	11	318±30	0.13±0.02	13	100± 7†
	Hypothyroid	4	137± 9.3†	0.033±0.004	5.9	179±23	0.08±0.02†	4.9	61±14
3	Euthyroid	5	246±17.6	0.050±0.004	7.6	152±25	0.17±0.02	6.1	36± 2
	Hyperthyroid	4	231± 6.9	0.044±0.003	10.2	231±15†	0.13±0.01	5.1	39± 2
	Hypothyroid	5	149±12.4†	0.029±0.002†	4.7	162±19	0.11±0.01†	5.3	48±10
6	Euthyroid	5	287±13.0	0.043±0.006	7.8	182±13	0.16±0.004	2.6	16± 0.7
	Hyperthyroid	5	227± 6.1†	0.038±0.001	7.3	191±22	0.12±0.008†	4.3	36±13
	Hypothyroid	4	132± 3.5†	0.024±0.001†	3.1	130±40	0.06±0.005†	1.4	23± 3†
12	Euthyroid	5	278± 3.5	0.040±0.002	6.0	150± 7	0.16±0.01	4.2	26± 1
	Hyperthyroid	4	234± 6.0†	0.041±0.004	6.5	159±10	0.12±0.01†	3.7	31± 2
	Hypothyroid	5	123± 2.5†	0.028±0.001†	1.9	68±12†	0.06±0.01†	2.3	38± 4†
24	Euthyroid	5	284±11.6	0.045±0.003	5.4	121±10	0.18±0.004	3.8	21± 2
	Hyperthyroid	5	251± 9.6	0.047±0.001	4.1	87± 8†	0.05±0.01†	2.3	15± 2†
	Hypothyroid	5	128± 6.5†	0.027±0.001†	1.7	63± 7†	0.05±0.02†	0.9	17± 1

* Mean±S.E.

† $p<0.05$.

TABLE XXIV

THE INFLUENCE OF THYROIDECTOMY AND OF THYROXINE DOSE AND DURATION OF TREATMENT ON THE BODY WEIGHTS OF RATS

(Thyroxine administration: 25 µg/kg/day for 12 weeks)

		Weeks of Treatment				
Thyroid State	Group	0	1	2	4	12
Euthyroid	1	135±5.1 (7)*	143±8.3 (7)	164±9.9 (7)	209±13.8 (7)	295±15.4 (6)
	2	124±2.4 (7)				272±10.3 (6)
	3	145±1.5 (8)				293± 7.8 (6)
	4	136±1.5 (8)				308±11.7 (6)
	5	140±0.7 (6)				298± 6.6 (6)
Hyperthyroid	1	134±2.2 (12)	137±1.9 (12)	140±2.2 (12)†	165± 2.7 (12)†	281±10.8 (6)
	2	129±2.1 (12)				252± 7.3 (6)
	3	148±2.3 (11)				284± 8.4 (6)
	4	148±3.1 (8)				288± 6.8 (6)
	5	145±1.7 (11)				266± 9.5 (6)
Hypothyroid	1	136±2.3 (10)	141±2.0 (10)	141±2.5 (10)†	143± 5.2 (10)†	141±13.1 (6)†
	2	121±5.5 (10)				138± 7.8 (6)
	3	152±3.2 (9)				166±12.5 (6)
	4	148±3.2 (6)				148±13.3 (6)
	5	147±2.4 (9)				148± 5.1 (6)

* Mean±S.E.

† $p<0.05$.

( ) indicates No. of animals used.

TABLE XXV

THE INFLUENCE OF THYROIDECTOMY AND OF THYROXINE DOSE AND DURATION OF TREATMENT ON THE HEART RATES OF RATS

(Thyroxine administration: 25 μg/kg/day for 12 weeks)

Thyroid State	Group	Weeks of Treatment 0	1	2	4	12
Euthyroid	1	492± 8 (7)*	520±10 (7)	509±11 (7)	483±3 (7)	413± 6 (6)
	2	522±14 (7)				422± 7 (6)
	3	510±15 (8)				418± 7 (6)
	4	459± 8 (8)				420± 6 (6)
	5	489±10 (6)				424±12 (6)
Hyperthyroid	1	390± 7 (12)	374± 6 (12)†	526±13 (12)	535±7 (12)†	492± 6 (6)†
	2	395± 7 (12)				488± 9 (6)
	3	383± 9 (11)				494± 6 (6)
	4	353±16 (8)				496± 6 (6)
	5	398± 9 (11)				516±10 (6)
Hypothyroid	1	386± 9 (10)	378± 6 (10)†	366± 7 (10)†	336±7 (10)†	314±16 (6)†
	2	389± 9 (10)				302±12 (6)
	3	380± 9 (9)				314± 9 (6)
	4	344± 4 (6)				322±20 (6)
	5	401± 7 (9)				318± 8 (6)

* Mean±S.E. (beats/min).
† $p < 0.05$.
( ) indicates No. of animals used.

TABLE XXVI

CATECHOLAMINE CONTENT OF RAT TISSUES IN DIFFERENT THYROID STATES

(Thyroxine administration: 25 μg/kg/day for 12 weeks)

Tissue	Thyroid State	No.	Body Wt (g)	Tissue Wt (g)	A μg/tissue	A μg/g	NA μg/tissue	NA μg/g	DA μg/tissue	DA μg/g
Heart	Euthyroid	30	293±5.0*	0.92±0.02	0.14	0.15±0.01	1.02	1.11±0.04		
	Hyperthyroid	30	274±4.4†	1.05±0.02†	0.13	0.12±0.01	1.23	1.17±0.06		
	Hypothyroid	30	148±4.9†	0.41±0.01†	0.05	0.13±0.02	0.64	1.56±0.04†		
Brain	Euthyroid	30	293±5.0	1.81±0.03	0.11	0.06±0.006	0.81	0.45±0.02	1.29	0.71±0.09 (12)
	Hyperthyroid	30	274±4.4†	1.85±0.02	0.11	0.06±0.007	0.85	0.46±0.02	1.61	0.87±0.07 (10)
	Hypothyroid	30	148±4.9†	1.63±0.02†	0.08	0.05±0.005	0.88	0.54±0.02†	1.32	0.81±0.11 (12)
Salivary Gland	Euthyroid	30	293±5.0	0.49±0.01	0.13	0.27±0.04	0.83	1.69±0.07		
	Hyperthyroid	30	274±4.4†	0.54±0.01†	0.16	0.29±0.06	1.15	2.13±0.10†		
	Hypothyroid	30	148±4.9†	0.25±0.01†	0.07	0.26±0.04	0.50	2.00±0.10†		
Adrenal Gland	Euthyroid	30	293±5.0	0.046±0.001	13.34	290±14	5.98	130±16	0.19	4.19±0.50 (15)
	Hyperthyroid	30	274±4.4†	0.047±0.001	13.68	291±20	7.10	151±24	0.32	6.82±0.30 (2)
	Hypothyroid	30	148±4.9†	0.028±0.001†	13.89	496±39†	8.96	320±46†	0.39	13.85±1.89 (13)†
Vas Deferens	Euthyroid	30	293±5.0	0.19±0.004	0.29	1.55±0.16	3.04	16±0.6		
	Hyperthyroid	30	274±4.4†	0.18±0.01	0.27	1.52±0.26	3.06	17±0.9		
	Hypothyroid	30	148±4.9†	0.09±0.01†	0.29	3.19±0.70†	2.25	25±2.0†		

* Mean±S.E.

† $p<0.05$.

( ) indicates No. of animals used in DA measurement.

TABLE XXVII

UPTAKE AND RETENTION OF ^{3}H-NORADRENALINE IN RAT TISSUES UNDER

DIFFERENT THYROID STATES

(Thyroxine administration: 25 μg/kg/day for 12 weeks)

Hour After Injection of ^{3}H-NA	Thyroid State	No.	Body Wt (g)	Heart			Brain			Salivary Gland		
				Wt (g)	Radioactivity		Wt (g)	Radioactivity		Wt (g)	Radioactivity	
					DPMx10^{-3}/tissue	DPMx10^{-3}/g		DPMx10^{-3}/tissue	DPMx10^{-3}/g		DPMx10^{-3}/tissue	DPMx10^{-3}/g
½	Euthyroid	6	295±15 *	0.94±0.05	979	1,042±59	1.59±0.09	2.5	1.6±0.13	0.49±0.02	153	312±50
	Hyperthyroid	6	281±10.8	1.07±0.03†	1,184	1,107±57	1.92±0.02†	3.1	1.6±0.31	0.56±0.02†	150	267±19
	Hypothyroid	6	141±13.1†	0.39±0.03†	296	759±57†	1.64±0.04	1.5	0.9±0.17	0.24±0.02†	53	221±20
3	Euthyroid	6	272±10.3	0.86±0.04	654	760±33	1.83±0.04	2.4	1.3±0.32	0.46±0.02	81	176±35
	Hyperthyroid	6	252± 7.3	0.98±0.03†	806	815±57	1.85±0.04	2.2	1.2±0.32	0.48±0.02	94	195±13
	Hypothyroid	6	138± 7.8†	0.39±0.02†	227	581±62	1.61±0.06†	1.6	1.0±0.17	0.22±0.02†	42	190±19
6	Euthyroid	6	293± 7.8	0.94±0.03	658	700±94	1.87±0.04	2.4	1.3±0.34	0.49±0.02	46	93± 6
	Hyperthyroid	6	284± 8.4	1.06±0.02†	681	641±49	1.81±0.08	2.2	1.2±0.23	0.52±0.03	58	112±16
	Hypothyroid	6	166±12.5†	0.44±0.02†	123	279±63†	1.76±0.03	1.4	0.8±0.11	0.27±0.02†	27	99± 4
12	Euthyroid	6	308±11.7	0.92±0.03	384	417±27	1.85±0.05	2.2	1.2±0.07	0.49±0.03	29	59± 5
	Hyperthyroid	6	288± 6.8	1.11±0.03†	414	373±18	1.81±0.04	1.4	0.8±0.07	0.58±0.02†	30	52± 1
	Hypothyroid	6	148±13.3†	0.40±0.03†	46	114±13†	1.58±0.06†	1.6	1.0±0.27	0.26±0.02†	12	48± 4
24	Euthyroid	6	298± 6.6	0.93±0.02	146	157±16	1.89±0.03	1.5	0.8±0.23	0.52±0.02	8.3	16± 2
	Hyperthyroid	6	266± 9.5†	1.01±0.05	285	282±23†	1.89±0.04	1.3	0.7±0.13	0.55±0.02	8.8	16± 3
	Hypothyroid	6	148± 5.1†	0.41±0.01†	16	39±10†	1.56±0.05†	0.8	0.5±0.06	0.25±0.01†	5.5	22± 2

* Mean±S.E.

† $p<0.05$.

TABLE XXVIII

UPTAKE AND RETENTION OF ^{3}H-NORADRENALINE IN RAT TISSUES UNDER

DIFFERENT THYROID STATES

(Thyroxine administration: 25 μg/kg/day for 12 weeks)

Hour After Injection of ^{3}H-NA	Thyroid State	No.	Body Wt (g)	Adrenal Gland Wt (g)	Adrenal Gland Radioactivity DPMx10^{-3}/tissue	Adrenal Gland Radioactivity DPMx10^{-3}/g	Vas Deferens Wt (g)	Vas Deferens Radioactivity DPMx10^{-3}/tissue	Vas Deferens Radioactivity DPMx10^{-3}/g
½	Euthyroid	6	295±15.4*	0.050±0.004	29	570±61	0.19±0.01	20	105±37
	Hyperthyroid	6	281±10.8	0.049±0.001	20	418±21†	0.21±0.01	15	71± 8
	Hypothyroid	6	141±13.1†	0.029±0.002†	7.3	252±38†	0.09±0.03†	6.4	71± 7
3	Euthyroid	6	272±10.3	0.044±0.002	16	364±13	0.18±0.01	13	70± 9
	Hyperthyroid	6	252± 7.3	0.048±0.002	19	387±41	0.17±0.01	12	69± 6
	Hypothyroid	6	138± 7.8†	0.023±0.001†	5.0	217±20†	0.11±0.02†	7.2	65± 6
6	Euthyroid	6	293± 7.8	0.043±0.001	16	365±43	0.19±0.01	11.2	59± 8
	Hyperthyroid	6	284± 8.4	0.048±0.002†	15	308±18	0.17±0.01	9.4	55± 4
	Hypothyroid	6	166±12.5†	0.030±0.002†	5.0	165±25†	0.11±0.02†	4.8	44± 8
12	Euthyroid	6	308±11.7	0.047±0.003	15	309±23	0.20±0.01	12	62± 3
	Hyperthyroid	6	288± 6.8	0.047±0.005	14	295±19	0.17±0.02	6.5	38± 5†
	Hypothyroid	6	148±13.3†	0.030±0.001†	2.4	81±12†	0.06±0.01†	2.2	36± 6†
24	Euthyroid	6	298± 6.6	0.047±0.002	9.1	194±23	0.18±0.01	4.7	26± 2
	Hyperthyroid	6	266± 9.5†	0.045±0.002	8.4	187±15	0.17±0.01	4.3	25± 1
	Hypothyroid	6	148± 5.1†	0.027±0.001†	2.1	78±10†	0.10±0.02†	1.3	13± 2†

* Mean±S.E.

† $p<0.05$.